transient nature and the number of leukocytes and eosinophils generally not so elevated, though Zamora and Fluhmann (21) reported cases from Chili with leukocyte-values up to 65,000 per cu. mm., and an eosinophilia of 77%.

The syndrome of Lohr-Leon-Kindberg (22), chronic pulmonary infiltration with eosinophilia, with its severe clinical aspect, is in marked contrast with the more quiet course of the tropical eosinophilia with its slight elevations of temperature.

As has already been emphasized, no parasites, neither eggs, v.g. eggs of *Bilharzia* were found in the sputa. As the clinical picture resembled somewhat the syndrome described by de Langen (6) in infection with *Strongyloides stercoralis*, we gave the same treatment, i.e. tartar emetic and the results were good; the attacks of asthma disappeared and the values for leukocytes and eosinophilics decreased, though an eosinophilia in the peripheral blood persisted. In Case 1 a recurrence followed after 2 months; treatment with mafarside (method of Weingarten) caused the symptoms to disappear completely; a result which has persisted up till now. In Case 2 no recurrence occurred and the blood-picture remained quiet.

It is known that tartar emetic does not kill the microfilaria but causes them to disappear from the peripheral blood (Das, Roy and Bose) (23) during several months. The good results obtained with arsenical compounds is remarkable, since it is known that salvarsan is ineffective in filariasis (Mayer) (24). The treatment with arsenical compounds is not always completely effective, as Weingarten saw recurrences after 3 or 4 years.

We did not succeed in determining the species of filariae found in our cases, but up till now only *Wuchereria bancrofti* has been found on Curaçao. The question why blood filariasis is not combined with pulmonary symptoms and hypereosinophilia and why the microfilaria did not occur in the blood in our cases must remain unanswered. We have pointed out already that in infection with macrofilaria local eosinophilia only occurs after the death of the worms (Hartz) (25).

However, we believe we have demonstrated the relationship between tropical eosinophilia and filariasis and reopened the discussion of the treatment of filariasis with arsenic.

SUMMARY

Three cases of tropical eosinophilia are described. In one case microfilaria could be demonstrated in the eosinophilic abscesses and infiltrate in the enlarged axillary lymph glands. The clinical symptoms disappeared after treatment with mafarside. In a second case there was myeloid metaplasia of an axillary gland, consisting almost exclusively of eosinophilic myelocytes. In a third case, with wide spread enlargement of the lymph nodes, at first a diagnosis of Hodgkin's disease was made; the clinical course and a second biopsy showed that it was a case of tropical eosinophilia.

In a 4th case which came at autopsy after an automobile accident hypereosinophilia of the blood in the liver was present; the spleen was enlarged and the subcapsular pulp contained enormous numbers of eosinophilic leukocytes and microfilaria. Eosinophilia was also found in the members of the family.

In accordance with the findings of Meyers and Kouwenaar, and of Bonne, the authors believe they have demonstrated the relationship between tropical eosinophilia and filariasis.

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ON THE EMPLOYMENT OF QUINACRINE HYDROCHLORIDE IN THE PREVENTION OF MALARIA INFECTIONS[1]

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We wish to record the results of certain experiments performed to ascertain the degree of protection against the acquirement of malaria infections afforded by various dosages of quinacrine hydrochloride (atabrine) when administered to patients inoculated with one or more species of malaria parasites at varying levels of inoculation intensity.

The drug employed was furnished through the courtesy of the Winthrop Chemical Company, and was received in original 1000-tablet containers bearing the identifying number BTO27.

Patients were inoculated by the application of various lots of insectary-reared *Anopheles quadrimaculatus* which had been experimentally infected with *Plasmodium vivax*, McCoy strain, or *Plasmodium falciparum*, Costa strain. After the mosquitoes had been used, their salivary glands were removed by dissection and examined for the presence of sporozoites.

The patients were white adult males presumably susceptible to malaria infections. They were kept under observation and daily blood smears were taken, for a period of at least six months subsequent to the termination of the course of quinacrine.

Experiment A. Single Inoculation with *Plasmodium falciparum*

Purpose. To ascertain the effectiveness of 0.1-gram doses of quinacrine hydrochloride, given on two discontinuous days a week, to protect against inoculations with *Plasmodium falciparum* on a single occasion, when the initial dose is given at varying intervals before or after the inoculation.

Materials and Methods. Anopheline mosquitoes derived from a lot infected with *P. falciparum* were divided into batches containing such numbers of mosquitoes that the presence in each of three infected specimens appeared probable. Fourteen white male patients were arranged in seven pairs, one of each pair being the test patient, the other the inoculation control. A batch of infected mosquitoes was applied to each test patient, and three days later the surviving members of each batch were applied to the corresponding control. The mosquitoes were then dissected and examined for sporozoites.

The test patients received 0.1 gram quinacrine hydrochloride twice, after breakfast and after dinner, on two non-successive days per week for four weeks. The day of the initial dose varied in relation to the inoculation in different patients, from the day before inoculation to the fifth day subsequent, in steps of one day. Daily blood smears from these patients were examined for a period of six months after the discontinuation of the quinacrine hydrochloride.

Results. The results of this experiment are presented in table 1.

Although all patients comprising the seven pairs were bitten by demonstrably infected mosquitoes, the control patients of pairs nos. 2, 4, and 7 did not develop an infection, and these pairs are consequently eliminated from further consideration. In view of the results from the control patients, it seems certain that the test patients in the first, third, fifth, and sixth pairs received infecting doses of sporozoites. The test patients of pairs 1 and 6 transitorily showed parasites after appropriate prepatent periods, the other two remained negative during a subsequent period of six months. The infection in the four control patients became clinically active.

Conclusions. The regimen of atabrine employed apparently prevented the development of infection in two test patients, but in two others the infection "broke through" but apparently was promptly extinguished by the continued administration of the drug before the parasitemia

[1] The studies and observations on which this paper is based were conducted with the support of and under the auspices of the International Health Division of The Rockefeller Foundation, in cooperation with the Florida State Board of Health and the Florida State Hospital.

crithidial or metacyclic form with a partial or a complete undulating membrane respectively. It is therefore obligatory to secure the cultural forms of flagellates in order to differentiate the two species of parasites from each other.

The cultural method is an important aid for the diagnosis of leishmaniasis and it should always be utilized, even though it may take from one to three weeks before positive laboratory findings can be secured. Positive cultures can be obtained from the peripheral blood as well as from bone marrow. It is desirable to inoculate six or more tubes of N. N. medium, each with 0.5 to 1 cc. of suspected blood.

In the present study, growth of flagellates was observed in about 5 days on N. N. medium that had been inoculated with bone marrow and in 16 to 26 days when such medium had been inoculated with peripheral blood. This is in harmony with the fact that the smears from the inoculum of the bone marrow revealed many Leishman-Donovan bodies; but in those made from blood, with one exception, no parasites were found. This probably accounts for the earlier cultural growth in the N. N. medium which had been inoculated with bone marrow. Whenever a speedy diagnosis is desired, the inoculum should be drawn from the bone marrow, even though positive cultures of the flagellates can also be secured from the circulating blood.

Since it is possible to secure positive cultures of *L. donovani* from both bone marrow and peripheral blood, the more hazardous splenic punctures can and should be avoided.

SUMMARY

1. Two exogenous cases of visceral leishmaniasis (kala-azar) are reported. These patients were Indian merchant seamen, who had apparently contracted their infections in Assam, India, and manifested their clinical symptoms in the United States. (The cases were referred to the hospital with a tentative diagnosis of malaria).

2. The chief clinical findings consisted of abdominal distress, high fever with an irregular temperature curve ("double crisis"), a markedly enlarged spleen and a slightly enlarged liver.

3. The laboratory findings showed leukopenia and anemia. All "presumptive tests" such as Napier's aldehyde or formol-gel, Chopra's antimony test, and Brahmacharis' serum globulin or "water test" gave positive results for kala-azar.

Smears obtained by sternal puncture from bone marrow showed many aflagellar forms of *Leishmania donovani*, both intracellularly and extracellularly. These parasites stained well with a combination of the Wright-Giemsa stains.

Positive cultures of flagellates were obtained from bone marrow on blood agar slants (N. N. medium). The flagellates grew readily within a week, were actively motile, and morphologically and culturally were indistinguishable from typical *Leishmania donovani*.

Positive cultures of *Leishmania donovani* were obtained likewise from the peripheral blood on slightly modified N. N. medium and showed a growth of flagellates after two weeks of incubation at room temperature.

The strain of *Leishmania donovani* isolated both from bone marrow and from the circulating blood has been subcultured monthly on blood agar slants. The flagellates grew luxuriantly, not only in the water of condensation, but also colonized on the slanted portion of the medium. The cultural forms taken stain readily and well.

4. Both patients were treated with stibamine glucoside and apparently were clinically cured when they were returned to India.

5. The fact that we have occasionally exogenous cases of leishmaniasis in the United States which have been diagnosed by specialists, suggests that similar, unrecognized cases may occur from time to time, and that it is increasingly important that clinicians and public health officers be on the alert for exogenous leishmaniasis.

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THE PLACE OF TROPICAL MEDICINE IN INTERNATIONAL HEALTH[1]

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The world crisis has already reached the stage at which plans are being laid for the future international organization to preserve peace and advance human welfare. The completed plans will undoubtedly give prominent place to provisions for effective collaboration between nations in promoting and protecting health, and will not overlook the outstanding problems of tropical medicine.

War, which might be regarded as a highly virulent infectious disease of society, is now in the final stages of its greatest pandemic. The best thought of our statesmen is being concentrated on future prevention. It is clearly recognized that to prevent war the causes of discontent and conflict must be diminished, while direct action will have to be made possible for application as a last resort. Likewise in the field of international health it is more important to remove conditions which give rise to disease, and to suppress it wherever it is first established, than to depend primarily on keeping pestilences from moving about after they have developed somewhere under neglect. Quarantine measures will still be necessary to curb the results of the limitations and failures of the broader international effort proposed, just as armed intervention may be needed at times to enforce peace when the more fundamental measures have been inadequately or belatedly applied. In the near future, however, we may expect to see the quarantine principle yield its dominant position to the more adequate and more friendly concept of helpful international collaboration in the suppression of disease wherever it still flourishes.

Tropical disease has been prominent in the history of international quarantine. Plague, cholera, yellow fever, typhus fever, and smallpox were the diseases specifically dealt with in the International Sanitary Convention of 1926. They included the pestilences which frightened nations into their early desperate efforts in health protection through ship quarantine. At least four of the five are classed as tropical diseases. In a later period tropical medicine contributed a large proportion of those brilliant discoveries and those successes in their application, which have made quarantine more scientific and effective and less burdensome, and have also given hope that most diseases can be effectively controlled at their points of origin.

It would be a poor outlook, however, if we should be satisfied with the application of these successes of the past and could expect only the application of existing knowledge and methods. The very rapidity of the recent advance in tropical medicine is indication that a great body of useful knowledge still lies ahead and invites discovery. The members of this Society should accept the challenge of the situation and should not fail to ward off the slump which might come with the cessation of the special demands of the war. The narrowing of certain research fields now specially favored should be more than offset by expansions in other directions where there has been unavoidable temporary neglect. If we fail to spur the efforts to accumulate new knowledge of tropical disease and to plan for its fullest application it may become difficult to justify this scientific Society. I have no fear, however, that tropical medicine in this part of the world will fail for lack of your interest.

Soon it will no longer be possible to excuse delay in disease control on the familiar ground that "there is a war on". It will again be possible, with reasonable support, to secure adequate personnel and materials. With the world war coming to an end, the war against disease will still be building up. What will be most needed for success is wise central planning and the giving of advice and assistance to countries with the greatest health problems of international interest. Here is where an effective and progressive international health organization comes into our picture. Without it health progress is bound to be spotty in distribution, reaching high levels in some countries and leaving others quite unable to cope with disease problems which may be unusually difficult and of great international importance. In fact

[1] Presidential Address before the American Society of Tropical Medicine, St. Louis, November 15, 1944.

disease itself may lower the power of the country to cope with it.

There are many new factors which increase the possibilities of rapid advance in the future control of tropical disease under adequate national and international organization. The now depleted civilian forces against disease will have many new recruits who have acquired experience and interest in tropical diseases while in military service in foreign lands, or while engaged in subsidized research related to the war effort. The present assistance to the teaching of tropical medicine in the medical schools should produce a succession of new doctors who already have interest and some fundamental knowledge in tropical medicine. The war-time revelation of the inadequacy of the instruction in tropical diseases has had a salutary effect. There is also a growing interest in securing greater facilities for post-graduate instruction and research in a few selected University Schools. This whole movement for adequate education and research deserves the fullest support.

It might be profitable to speculate as to the future of malaria control. This disease has already been openly threatened with a war of extermination in the United States after the world war ends. The recent demonstration in Brazil that under certain circumstances a dangerous species of anopheline can be exterminated throughout a wide area by systematic measures, has led to the commencement of similar attempts in South America and North Africa even before the end of the war. The war-time experience in West Africa has emphasized the need for thorough suppression of anophelines throughout important malarious areas and in vital ports by equally comprehensive and vigorous methods. Success in these ventures should be possible when the manpower and materials and trained leadership become available; especially as larvicidal substances more effective than paris green will probably be at hand.

The planning of the critical first demonstrations of complete control in difficult situations should be thoroughly done and preferably with central guidance, perhaps by a commission created by the future international health organization. We need a central strategy board which can map the places of danger, measure the risks, and make a time schedule for the campaign. It could also estimate the costs in terms of personnel and materials, and propose ways of allocating them.

Such a commission could decide what quarantine and control measures are necessary to prevent dangerous species of anophelines from invading the malaria-free islands of the Pacific, or again crossing the Atlantic to re-establish themselves in South America. It could determine the order of preference, in relation to danger and need, of projects for controlling malaria in the infected countries.

The review of the world situation with regard to typhus fever by an international health organization or a special commission appointed by it, is overdue. The louse-infestation which permits epidemics of typhus should be eliminated by methods now well understood. Living conditions and standards of hygiene should of course be fostered which would be incompatible with lousiness, but in addition the more rapid measures of delousing by simple powdering should be systematically applied under the direction of the health authorities. The body louse should be easily exterminated in any threatened area, and then there should be no fear of serious epidemics of typhus. Such a commission could also decide the conditions under which vaccination against typhus should be utilized in addition. Certainly we should cease to regard certain regions as permanent endemic centres of typhus, when there are effective methods for rendering communities non-infectible at low cost. What is needed is the stimulation and guidance of a central agency in which each of the countries involved participates.

In the case of yellow fever, that most typical of tropical diseases, there will be opportunity for still further advances toward complete control. The past decade has seen marked improvement in methods, but a comprehensive international system of prevention is still to be devised. Only through a common plan can all the interested countries protect each other and themselves. Even our present knowledge should make it possible to devise and carry out a yellow fever program which would be a model for international disease control.

With yellow fever firmly entrenched in animals and mosquitoes of the tropical forest in South America and Africa complete extermination of the disease from the world has had to be indefinitely postponed. It must at least await new discoveries not yet foreseen. In the meanwhile local extermination of the urban disease through elimination of the vector *Aedes aegypti*, and the confinement of the infection to the endemic areas of jungle yellow fever by barrier vaccination can most certainly be accomplished. Moreover, in-

ternational quarantine regulations relating to yellow fever can be modernized and liberalized so that threatened countries will receive all possible protection while restrictions on infected countries can be safely reduced if they keep their seaports and airports and surrounding regions noninfectible.

It would seem that an adequate international system of yellow fever control would include five essential parts which I shall enumerate:

1) There should be frequent and adequate surveys of all endemic and suspected regions, with immunity tests of men and animals on a sampling basis and with adequate mapping and statistical analysis. Only by constant vigilance can we ascertain where precautions are needed and avoid being taken by surprise from an unexpected quarter. An international organization is necessary for the even exercise of such a function for the benefit of all nations.

2) Barriers should be erected around endemic areas of jungle yellow fever through vaccination of the inhabitants and the extermination of *Aedes aegypti* if present in neighboring towns. The object would be twofold—to protect the local people themselves and to keep the disease from wandering out in infected persons and starting widespread epidemics.

3) Infected or threatened cities, and particularly seaports and airports, should be made completely non-infectible by the extermination of *Aedes aegypti*. If this is done and there is constant vigilance to prevent the return of this insect, it would be impossible for yellow fever to spread if introduced from the jungle.

4) Adequate international quarantine provisions would be needed as a third line of defense to prevent the conveyance of yellow fever from port to port or country to country in case it should pass the barrier around the jungle area and find a port in which *Aedes aegypti* has not been fully suppressed. Yellow fever quarantine measures are receiving special attention in the revision of the International Sanitary Convention of 1926 and the International Sanitary Convention for Aerial Navigation of 1933. Convinced that the Office international d'Hygiène publique, on account of the war situation, is not now in a position effectively to carry out the duties assigned to it under these conventions, the Council of the United Nations Relief and Rehabilitation Administration recently approved in principle proposed amendments bringing the conventions up to date and providing for their administration for the time being by UNRRA, but without prejudice to the return of the functions to the Office international d'Hygiène publique. As soon as the new conventions carrying the amendments have been signed or acceded to on behalf of ten or more governments, they will come into force. For the immediate administration of the conventions and particularly for the determination of endemic areas for purposes of quarantine, UNRRA's Technical Committee on Health has set up an Expert Commission on Quarantine which will cooperate closely with the Pan-American Sanitary Bureau.

5) International facilities for promoting education and research in the health field and for exchanging knowledge and experience, is the fifth of the essential parts of the program for the control of yellow fever, and it would apply equally to any other important disease problem. Means must be found to encourage and enable health officials and medical scientists of the interested countries to study and confer wherever the opportunities are most favorable. They should meet and investigate where disease is active in its natural environment and also at centers where medical science is reaching its highest development. Only when research is promoted and knowledge freely exchanged shall we acquire a body of participating health administrators and scientists able to make a success of the international health organization we envisage.

Classic typhus, cholera, and yellow fever are among those diseases dependent for survival on correctible conditions. In fact these diseases have disappeared completely from many countries which have become practically non-infectible, although once ravaged. We know what would have to be done to eliminate any one of these diseases from the people of any locality. It follows that if we are really interested in having a healthier world, as well as one safer from war, we should begin thinking in global terms. We should organize our forces to strike wherever preventable disease is prevalent, and to apply by preference those measures which lead steadily to conditions incompatible with its persistence. Sometimes this means providing pure water for twenty-four hours in the day; sometimes more soap is needed and better housing and improved economic conditions for the groups hardest hit. There are always, however, additional scientific methods of direct attack to hasten victory. It is obvious that the best effort of each nation as well

as of the whole group will be required under ideal cooperation. If the vulnerable diseases, including especially infectious and nutritional diseases, among which many are classed as tropical, are to be thoroughly suppressed in our time, there must soon be established an effective cooperative world health organization. The Health Organization of the League of Nations made a good beginning after the last World War. UNRRA should add valuable experience in its developing war-emergency health work. The time is surely ripe for setting up the ideal permanent international health organization.

My message to the Society, as its retiring president, is to urge that each member look beyond his own specialty within the broad field of tropical medicine and beyond the geographical boundaries of the lands plagued by the diseases of his dominant interest and contribute to the thought and statesmanship which will be needed for setting up a suitable and acceptable world health organization. We can then hope soon to reach a situation in which every country, no matter how small or how poor, can handle its own problems with such central help as may be necessary and can cease passing pestilences to its neighbors.

No country can live to itself in disease prevention any more than in political relations and commerce. We must reach a state of knowledge, a state of organization and a state of mind which permit our recognizing that all nations are allies in the fight against disease, and that the failure of one is the failure of all.

THE PRESENT STATUS OF TROPICAL MEDICINE AND SOME FUTURE PROBLEMS[1]

EDWARD B. VEDDER*

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Individuals, Societies and Nations are born, grow into a vigorous manhood, become aged and die. Some organizations possessing unusual vigor live a long time, like the Catholic Church which has outlasted many nations; and as Macaulay wrote "She may still exist in undiminished vigor when some traveller from New Zealand shall, in the midst of a vast solitude take his stand on a broken arch of London Bridge to sketch the ruins of St. Paul's."

Is there any reason to suppose that our Country will last as long as the Egypt of the Pharaohs or as long as ancient Rome? It certainly will not last very long under officials who wish to arrogate all powers to themselves and to regulate the lives of every one of us.

This country was founded on the idea that only the liberties of the people were important, and that the State existed solely to serve them. That State was best that governed least. How different from the present time when it is proposed to subject the entire medical profession to the whims of a purely political appointee.

This is not a political speech, and I mention these facts merely to indicate a condition that threatens the health of our medical societies, including those in which we are chiefly interested here, The American Society of Tropical Medicine and the American Academy of Tropical Medicine. All those who are interested in any phase of Scientific Medicine should put themselves on record in opposition to this political brand of socialized medicine.

This Academy of Tropical Medicine is still in its lusty childhood. The fact that the Foundation of Tropical Medicine is finally functioning, and that the teaching of Tropical Medicine in various Medical Schools is now endowed is a sign of healthy development (1). The American Society of Tropical Medicine is considerably older and appears to be in its prime.

Tropical Medicine was born in this Country as the result of our acquisition of the Philippines and received its preliminary education there and in Panama. At present it is taking its college course in our South Sea Island adventures. Tropical Medicine in the British Empire is considerably older, and even has a history as typified in the able and interesting History of Tropical Medicine by Scott published in 1939.

Abandoning these figures of speech, The American Society of Tropical Medicine was organized March 9, 1903. In 1913, just ten years afterward there were 121 active members. In 1921 the membership had declined to 108, and possibly the only reason the society survived at that point was the able editorial policy of the American Journal of Tropical Medicine. By 1935 the membership had increased to 429, and at the present time in 1944 it is approximately 1,200.

This sudden growth, together with the multiplication of Textbooks of Tropical Medicine, and the increasingly serious teaching of Tropical Medicine is undoubtedly due to the encounter with tropical diseases in the Pacific Islands, and to our fear that many of these diseases may be introduced into the United States by our returning soldiers and sailors.

Since it is a common experience for these enthusiasms to evaporate and for society memberships to decline after a war is over, our first problem is how to maintain that interest. One method is to insist that teaching of Tropical Medicine must be continued in our medical schools as a regular subject (2).

Before the War there were courses of tropical medicine in certain schools, but they were stepchildren. In the School with which I was connected, it was an elective, and I flattered myself that the course was fairly interesting because I usually got about half the class. We insist that it must be a required subject in all medical schools. Practically all graduates are going to have an opportunity to see and treat certain tropical diseases, while very few of them will ever practice surgery.

[1] Presidential Address delivered at the 11th Annual Dinner Session of the American Academy of Tropical Medicine, St. Louis, Mo., November 15th, 1944.

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I also hope that all of the Pacific Islands that do not belong to England or China will be kept permanently by the United States. If we are capable of learning by experience, it should be evident that we cannot entrust these islands to any other Power. We have purchased them at the expense of much blood and treasure. Even in the Philippines, although we may give them their freedom, it must be understood that we will keep Corregidor, Cavite, and keep troops stationed at key positions. The Filipinos must know by this time that they can never defend themselves, and only a great naval power can do so.

If we keep the Pacific Islands, we will be compelled to maintain garrisons in them and tropical diseases will become a continuous problem. I trust that the solution of these problems will lead to the formation of more Army Boards for the study of Tropical Diseases like the board in Manila, first established by Strong, where so many investigations on Tropical Diseases were carried out. In any case, our Army and Navy surgeons will undoubtedly make large additions to our knowledge, and we may expect that they will become members of our societies and so maintain their growth and increase the interest of our meetings.

What of the danger of importation of Tropical Diseases into the United States? I am not one of those who think that this danger is great. Since both malaria and amebic dysentery, or amebiasis, if you prefer that term, are prone to relapse after apparent cure, it may be assumed that some of these cases will be returned to the United States. This need not alarm us. In the first place, the services will undoubtedly do the best they can to eliminate these infections before the men are discharged into the civilian community. So far as the Army is concerned, a special treatment center was opened on September 1, 1944, at Moore General Hospital, North Carolina, for the treatment of malaria and other tropical diseases. The work is being supervised by the Tropical Disease Branch of the Medical Division of the Surgeon General's Office. I am not so familiar with the Navy, but I assume that they are at least as active.

But even if men are discharged with these infections, it is only fair to state that they are no novelty to any part of the United States. Meleney (3) has shown that in 1934 the mortality from malaria in 13 Southern States was 3,900, as much as the combined figures for typhoid and diphtheria. Our experience with amebic dysentery in Chicago hotels in 1933 is not yet forgotten, and Craig, Meleney, Faust, and other of our members have shown how widespread is this disease in the United States, from Virginia to California, and from Michigan to Florida and Louisiana. But if a few more cases are added to our endemic infection, at least we can say that we know more about these diseases than formerly; and that more physicians have been trained in diagnosis and treatment than ever before, largely owing to the efficient teaching of Tropical Medicine to numbers of physicians by Strong, Mackie and others at the Army Medical School, and at the School of Tropical Medicine headed by Faust at Tulane University.

For amebiasis, we have three more or less specific remedies, emetine, arsenicals and quinoline derivatives. And although we may be somewhat short of quinine at present, we have learned that synthetic atabrine (4) is even better, since, when properly administered, there is evidence of curative effect in falciparum malaria, and, further, that it will prevent consistently the development of falciparum malaria, the only form of malaria responsible for most of the deaths.

It remains as one of our future problems to discover a drug that will destroy the sporozoites of all forms of malaria. Will anyone believe that this is impossible to our synthetic chemists in view of what they have already accomplished? When that drug is achieved, we will be in a position to prevent infection by mosquitoes with proper prophylactic doses, and the final conquest of malaria will become a possibility.

What diseases in addition to malaria and amebiasis can be introduced among us? We think first of mosquito-borne diseases like yellow fever and filariasis. Remembering the introduction of *Anopheles gambiae* from Africa by airplane, it seems easy to bring yellow fever in the same way. Proper precautions are already being taken against this danger. As described by C. L. Williams (5) airplanes from danger spots are repeatedly fumigated to prevent the importation of infected *Aedes* mosquitoes. Individuals who fly from infected ports are followed by the various health officials after they land for nine days after the last possible exposure to yellow fever, to be certain that they do not develop the disease after landing.

Filariasis due to *Wuchereria bancrofti* is more likely to be transmitted here, because the parasite may be transmitted by a considerable number of both genera and species of mosquitoes; because a number of infected cases have already been re-

turned to the United States, and because we have already had one endemic focus in the Carolinas. Although this infection did not spread and has probably now died out, we have no proof that other cases introduced will be so limited. At least we have no reason to dread the resulting elephantiasis which apparently occurs only in very heavy infections. Filariasis was found to be rather widely disseminated in the Philippines, but there was very little elephantiasis, nor did I ever hear of a white man developing elephantiasis.

Nevertheless, one of our most urgent future problems is to discover a reliable cure for this infection. Nor is this a hopeless investigation. We already have the clue. Wright and Underwood (6) reported in 1934 that Fuadin destroyed *Dirofilaria immitis* or heartworm of dogs. Culbertson and Rose (7) reported in 1944 that cotton rats naturally infected with filariasis have been successfully treated with stibamine glucoside. It has also been shown that this drug is well tolerated by man in comparatively large doses. Still more recently Brown (8) has treated twelve human cases of filariasis (*Wuchereria bancrofti*) with lithium antimony thiomalate. The treatment reduced microfilaria counts from 85–100 per cent, and the reduction was maintained 4–5 months, so that presumably a corresponding number of adult worms were killed. Since schistosomes are also destroyed by antimonial preparations, it appears probable that after some experimentation we shall shortly find the ideal antimony compound for the destruction of the various filariae parasitic in man.

Many diseases that are common in the tropics cannot spread here for various reasons: first, because the vectors do not live here, which excludes the importation of human trypanosomiasis, (excepting Chagas' disease) leishmaniasis, kala azar, and probably schistosomiasis. Our food habits exclude *Paragonimus* since we do not eat raw crabs or crayfish, in which the encysted cercariae live. For similar reasons, *Clonorchis sinensis*, *Fasciolopsis buski* and other parasites will fail to spread. Our methods of sewage disposal, and the fact that all soldiers usually wear shoes, mean that relatively few hookworms and other intestinal parasites will be imported or transmitted here. Few white men acquire yaws. Gonorrhoea is now cured in a few days by combined sulfa compounds and penicillin. Even syphilis is now yielding rapidly to penicillin, though as yet we do not know how permanent these results will be. At least we may be assured that there will be no great influx into this country of any of the venereal or treponematous infections.

One problem we have had before us for forty years; I refer to plague. Plague, originally concealed in San Francisco, was eventually suppressed there, but before that time the ground squirrels had become infected. This wild rodent plague has resulted in two serious outbreaks of human plague in California; in 1919 with 13 cases and in 1924 with 32 cases and 30 deaths. This is bad enough, but rodent plague has been extending steadily from State to State until in 1940 ten States were infected: California, Oregon, Washington, Idaho, Montana, Nevada, Utah, Wyoming, New Mexico and Arizona (9). Its spread Eastward is probable. We already have Rocky Mountain spotted fever in Virginia, Maryland and the District of Columbia. Plague has been demonstrated in fourteen species of ground squirrels; in flying squirrels, chipmunks, field mice, wood rats, kangaroo rats, the cotton rat, and in rabbits as well as in domestic rats. This is not guesswork. Experimental inoculations with fleas, lice, and ticks removed from these naturally infected rodents have resulted in plague in laboratory animals. We are apparently helpless in the face of this problem. The public health authorities have been working all these years to eradicate plague in wild rodents by means of poisoned baits, trapping and other usual methods, and in spite of these efforts the spread of rodent plague has been continuous.

I can offer no solution to this problem and merely state it. It is evident that we must be prepared to diagnose and treat the cases of human plague that may occur at any time. Fortunately there is some reason to believe that we are better prepared to treat human plague. Concentrated antiplague serum has been shown to be four times as potent as the plague serum formerly used, and animal experiments have indicated that when plague infected they may be saved by the administration of sulfathiazole even after septicemia has occurred.

Infectious hepatitis is another important problem. This disease has affected troops in Northern Africa and the Levant. It has a small but definite death rate, but as individuals are ill from four to six weeks, the morbidity rate is very high. We need not worry about importation of infectious jaundice for the disease is not limited to the tropics, but can be seen almost daily during the season in any of our larger hospitals in the United States.

A considerable number of cases of infectious hepatitis were produced in officers and men inoculated with certain strains of yellow fever vaccine. Investigation led to the conclusion that these cases were caused by the serum with which they were mixed. It had nothing to do with immunization to yellow fever *per se*, and other lots of yellow fever vaccine were innocuous except as they produced immunity to that disease. The fact that only certain lots of serum contained the infectious agent of hepatitis, indicates that infectious hepatitis is caused by a virus which is present in the blood. It has been reported that the virus of this form of hepatitis has been cultivated on the developing hen's egg from the duodenal fluid of patients (10). Sams (11) states it is known that the virus is in the blood, particularly in the pre-icteric state, and that it can be transmitted to others by direct inoculation of the blood. A study has been made of the epidemiology of one outbreak in Northern Africa, and a commission has been working on the problem of how the disease is transmitted. It seems probable that such a virus can only be conveyed from person to person by biting insects. Sams suspects *Aedes aegypti* because "after an unusual so-called wind-borne flight of these mosquitoes into an area in which certain troops were located, an outbreak of infectious hepatitis subsequently developed." If this is correct the disease is transmitted at least part of the time by the same mosquito that is responsible for yellow fever and dengue. I have some doubt that this is the sole method of transmission. The disease occurs in the United States in areas where *Aedes* hardly if ever occurs. It is possible that in these localities it is transmitted by other species of mosquitoes, though biting flies and ticks must be considered and proven or excluded. It is urgently necessary to discover the method or methods by which this disease is transmitted, for only in this way can it be prevented.

At present such cases can only be treated by high protein, high carbohydrate, low fat diets. No medicinal agent is known. While we have had little success in the past in developing specific therapy for virus diseases, the effort should not be abandoned. Who would have supposed ten years ago that we would have agents at our command like the sulphones and penicillin? There is also the possibility of developing either an anti-serum or a vaccine.

Another problem before us today is that of the vitamins. As the result of the investigations of a long series of workers from all countries, the etiology of the deficiency diseases, scurvy, beriberi, pellagra and rickets was elucidated. Starting with Williams (12) who synthesized thiamin, a long series of vitamins have been synthesized. This has led to cheap production and easy sale.

From the earlier investigations we were accustomed to think of deficiency diseases as caused by the lack of specific vitamins. Scurvy was due to the deficiency of ascorbic acid; beriberi to the deficiency of vitamin B_1 or thiamin; pellagra was caused by the deficiency of nicotinic acid. This conception was developed from experimental work on animals fed synthetic diets, often deficient in a single vitamin. But from the beginning none of these animal diseases was quite the same as disease in man. So far as human beings are concerned, deficiency diseases arise from the use of more or less definite diets which are not only monotonous, but can quite readily be shown to be lacking in several vitamins. Scurvy as it occurred among sailors for some two hundred years was produced by a diet of salt meat and sea biscuit, and this diet is not only deficient in ascorbic acid but also in thiamin producing edema and sudden cardiac death, and in vitamin A resulting in night blindness. There were other probable deficiencies.

The complete clinical picture of pellagra, as we have learned from the work of Spies and his collaborators is not only principally caused by the lack of nicotinic acid but by the additional deficiencies of thiamin, riboflavin pyrodoxin and pantothenic acid. The same thing is true of beriberi, usually produced on a rice and fish diet which also has multiple deficiencies.

What is worse, in addition to outright deficiency diseases, it has been found that the deprivation of any of these elements leads to vague symptoms and a substandard state, long before the corresponding disease is fully established.

Although this work is sound, and vitamins are real chemical compounds that are absolutely essential dietary ingredients, the popularization of this work has led to a vitamin craze. If we read a newspaper our eyes are assailed, and if we listen to a radio program our ears are insulted with the grossest misstatements about the absolute necessity of taking John Smith's vitamin formula if we would enjoy good health and have the proper sex appeal. As the result of all this organized advertising, in 1943 the public spent 179 million dollars for vitamins. How much better and

cheaper to buy the foods containing them (13). There are almost certainly other vitamins than those already identified. We have been in the habit of instructing patients to eat several eggs a week. It has recently been shown that we are quite unable to synthesize the simple methyl group CH_3, which must be furnished in our food. The only amino-acid that contains a methyl group is methionine, and diets built upon proteins that do not contain methionine will not support life in experimental animals. But they will begin to grow again rapidly if choline is added to the diet because choline contains three methyl groups. The yolk of egg consists largely of lecithin, and the main ingredient of lecithin is choline. Thus we can supply plenty of methyl groups in eggs, and they cannot be purchased in the drug store. Oil of wintergreen is methyl salicylate, but investigation has shown that these simple methyl compounds cannot be utilized by the body.

While we find many poorly nourished patients in our hospitals, outright deficiency diseases are unusual in most sections of our country. It is preferable to feed these patients proper diets rather than to give vitamin mixtures. These should be reserved for the patients who, for one reason or another, are compelled to eat a limited one-sided diet. In the hospital where I work at present, instead of administering ascorbic acid, the physician is compelled to order orange juice, even if the orange juice costs more than the synthetic vitamin. This for the very good reason that when the patient leaves the hospital, he will not go to the drug store to buy ascorbic acid, but to the grocery to purchase oranges. Fresh orange juice not only has half a milligram of ascorbic acid per cubic centimeter, but has many other valuable food ingredients including sugar and two other vitamins.

It should be the duty of all physicians, including ourselves, to instruct patients what foods must be supplied in order to furnish proper nutrition. Fortunately most of these foods are not rationed.

There are many other problems that I would like to touch upon did time permit. Typhus is such a problem and its prophylaxis and treatment require experimentation (14).

You will all understand that I have made no attempt to be encyclopedic in this address. There would be no purpose in such a discussion before a gathering composed of experts in Tropical Medicine. I would have preferred to be silent and listen to some one else of this group. Since I have been compelled by the office to speak, I have tried to express a point of view that would be both scientific and common sense. I hope that most of you will agree with me.

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THE COMPLEMENT FIXATION TEST IN THE DIAGNOSIS OF YELLOW FEVER [1]

Comparative Value of the Serologic and Histopathologic Methods of Diagnosis

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Hughes (1), in his study on the precipitin reaction in yellow fever, observed that there was no parallelism between the virus content and the precipitinogen content of a serum; the amount of precipitinogen in the serum, however, varied roughly with the severity of the infection, and he suggested, therefore, that the precipitinogen may represent a foreign protein arising from tissues damaged by the action of the virus.

An analogous situation obtains as regards the complement-fixing antigen of yellow fever (1, 2). If the antigen is a product of cellular damage provoked by the virus, it is conceivable that the liver, which in fatal cases of infection is almost invariably, and frequently severely, involved, might constitute one of the sources of origin of the antigen. If this be true, there arises the question of the specificity of the antigen; i.e., is the antigen the result of subtle chemical alterations produced within the liver cells by the virus of yellow fever *per se*, and only by the virus, or is it elaborated by some pathologic process whose end result may be difficult or impossible to distinguish microscopically from that of yellow fever. Information on the latter point is of some consequence, since it was previously shown (2) that the complement-fixing antigen appears in the blood early in the course of the experimental disease and that its demonstration may be a more accurate means of diagnosis than is microscopic examination of the liver. In the present communication, therefore, we wish to report on observations concerning the specificity of the antigen and the comparative value of the serologic and histopathologic methods in the diagnosis of experimental yellow fever.

MATERIAL AND METHODS

Tannic acid. Animals were inoculated subcutaneously with freshly prepared 5 per cent solutions of tannic acid (Baker's C.P.) in distilled water. The dosage, which ranged from 50 to 500 mg. per kg. of body weight, is given under each experiment.

Serum antigen. Serum specimens to be examined for the presence of antigen were diluted 1:5 with physiological salt solution and inactivated in a water bath for 30 minutes at 60°C.

Liver antigens. The technique previously used for brain antigens (3) was applied to the preparation of liver antigens, except that isotonic salt solution without serum was used as the suspending medium and the supernatant obtained after the final centrifugation was filtered through a Seitz EK pad.

Complement-fixation tests. Examination of tissues for complement-fixing antigen was conducted according to the method previously described (2, 3). Serial twofold dilutions were made of the antigen preparations, beginning with 1:5, and tested against a 1:4 dilution of pooled immune monkey serum of known fixing potency and a 1:4 dilution of pooled normal monkey serum. Monkey or marmoset sera known to contain the antigen were included in every test as controls.

Microscopic examination of tissues. Small portions of liver were fixed in 10 per cent formol-saline and embedded in paraffin after dehydration; the cut sections were stained with hematoxylin and eosin.

To check on the presence of the intranuclear inclusions described by Nicolau et al. (4), blocks of liver were fixed in Duboscq-Brasil and the sections were stained by Mann's method.

[1] The work on which these observations are based was carried out with the support and under the auspices of the Serviço de Estudos e Pesquisas sôbre a Febre Amarela, which is maintained jointly by the Ministry of Education and Health of Brazil and the International Health Division of The Rockefeller Foundation.

[2] From the Serviço de Estudos e Pesquisas sôbre a Febre Amarela, Rio de Janeiro, Brazil.

EXPERIMENTAL

The lesions occurring in the liver in yellow fever have been described by Councilman (5), da Rocha Lima (6), Hudson (7), Magarinos Torres (8), Klotz and Belt (9), Laemmert (10), and others; the microscopic findings considered as classic for yellow fever have recently been succinctly summarized by E. Villela (11). Considerable experience is required to make a diagnosis of yellow fever on the basis of the microscopic examination of the liver alone, for example of human specimens secured by viscerotomy, since the lesions produced in the liver by the yellow fever virus closely resemble those of acute yellow atrophy and possess several features in common with lesions produced by other viruses (9). Unquestionably there exist other conditions whose effects on the liver may be confounded with those of yellow fever; thus Belt (12) has reported that persons with fatal burns which had been treated with tannic acid were found at autopsy to present severe liver damage, with midzonal necrosis, Councilman lesions, and intranuclear inclusions, a pathologic picture practically indistinguishable from that seen in yellow fever. Wells, Humphrey, and Coll (13) and Erb, Morgan, and Farmer (14) have recently reported similar observations on persons with fatal burns treated with tannic acid, and the toxic action of this agent on the liver has been demonstrated in mice, rats, guinea pigs, rabbits, and goats (15–18).

Because of this close similarity between the lesions provoked by tannic acid and by yellow fever virus, experiments were undertaken to determine (a) the extent to which the microscopic picture produced in the liver by the acid might be confused with that evoked by the virus and (b) whether the identicalness of the microscopic lesions in the two conditions is attended by the presence in the blood of identical complement-fixing antigens. Twenty-one Brazilian marmosets (*Callithrix jacchus*) and two monkeys (*Macaca mulatta*) were used in the tannic acid work, and the results were compared with those obtained in 10 marmosets and four monkeys infected with yellow fever virus.

Ten marmosets (nos. 1–10 in table 1) were inoculated subcutaneously with tannic acid in doses varying from 150–500 mg. per kg. of body weight. Nine of the animals died suddenly within 48 to 72 hours after inoculation, and the tenth animal was found dead on the seventh post-inoculation day; none of the animals had shown any obvious signs of illness. It was possible to obtain sufficient serum for serologic examination from only four of the animals post mortem, but the livers of all were removed for serologic and histologic study. Microscopic examination of the livers revealed that the acid had produced relatively little damage. This was attributed to the rapidity with which injection of a single large dose of the acid resulted in the death of the animal; and to allow the destructive process to act over a longer time, a second series of animals (Marmosets nos. 11–21) was inoculated repeatedly with smaller doses of the acid. Six marmosets were inoculated subcutaneously with doses of 100 mg. per kg. of body weight and five with 50 mg. per kg. of body weight. Eight of the animals were given daily injections up to the time of death, which occurred from 4 to 7 days after the first injection; in three animals, interposition of a rest period of 4 days between the first and the subsequent injections lengthened the survival time to 8 to 12 days.

In addition to the marmosets, two monkeys were inoculated. A dose of 250 mg. per kg. of body weight was used, and two injections were given. To one monkey the second dose was administered 10 days after the first and to the other the second dose was given 13 days after the first; the animals died on the twelfth and the fourteenth day, respectively, after the first injection.

Since the complement-fixing antigen was always found present in the blood of marmosets and monkeys succumbing to yellow fever virus infection (2) the animals that had been treated with tannic acid were bled when moribund; in several instances blood was taken post mortem. Several animals were bled at intervals during the course of the injections in order to follow the appearance of antigen, if any were produced.

The results of the serologic and histologic studies are presented in table 1, in which the animals are listed according to the dosage of tannic acid given.

It will be observed that the sera of 17 animals were tested for the presence of antigen capable of giving fixation with yellow fever immune serum, and all were found to be negative. Likewise, the livers of all 23 animals were found to contain no demonstrable antigen. (Acute-phase yellow fever sera, used as antigen controls, gave positive fixation tests in dilutions as high as 1:640 or 1:1280).

TABLE 1

Serologic and histopathologic examination of tissue from animals inoculated with tannic acid

Animal	Tannic acid inoculated: Dosage	Tannic acid inoculated: Number of injections	Tannic acid inoculated: Total amount injected	Day of death postinoculation*	Results of examination of postinoculation sera†: Serum taken	Results of examination of postinoculation sera†: Yellow fever C.F. antigen	Results of examination of liver tissue: Yellow fever C.F. antigen	Pathologic changes‡: Councilman lesions	Pathologic changes‡: Intranuclear inclusions: Classic type	Pathologic changes‡: Intranuclear inclusions: Nicolau type	Pathologic changes‡: Fatty degeneration	Remarks	Histopathologic diagnosis
	mg./kg.		*mc.*		*day*								
Marmoset													
1	500	1	175	2nd	2nd	none	none	+	−	+	−	Slight necrosis, congestion	Negative for yellow fever
2	500	1	170	2nd	2nd	none	none	+	+	+	+	Slight necrosis	Positive for yellow fever
3	500	1	165	2nd	X	X	none	+	−	+	+	Slight necrosis	Negative for yellow fever
4	500	1	144	2nd	2nd	none	none	+	−	+	+	Slight necrosis, necrobiosis	Negative for yellow fever
5	500	1	135	2nd	2nd	none	none	+	+	+	+	Few necrotic cells, abundant inclusions of both types	Positive for yellow fever
6	250	1	57	7th	X	X	none	+	+	+	+	Slight necrosis, abundant inclusions of both types	Positive for yellow fever
7	250	1	55	2nd	X	X	none	+	−	−	+	Few necrotic cells, severe fatty degeneration	Negative for yellow fever
8	250	1	40	3rd	X	X	none	+	−	−	+	Slight necrosis, severe fatty degeneration, congestion	Negative for yellow fever
9	150	1	40	3rd	X	X	none	+	−	−	+	Few scattered necrotic cells, central necrosis, congestion	Negative for yellow fever
10	150	2	80	2nd	X	X	none	+	+	−	−	Slight necrosis, necrobiosis, many inclusions	Positive for yellow fever
11	100	4	120	4th	3rd, 4th	none	none	+	+	−	+	Moderate necrosis, severe fatty degeneration, few inclusions	Positive for yellow fever
12	100	4	100	4th	3rd, 4th	none	none	+	+	+	+	Moderate necrosis, many inclusions of both types, severe fatty degeneration	Positive for yellow fever
13	100	4	100	4th	3rd	none	none	+	+	+	+	Moderate necrosis, few inclusions	Positive for yellow fever

TABLE 1—*Concluded*

Animal	Tannic acid inoculated: Dosage	Tannic acid inoculated: Number of injections	Tannic acid inoculated: Total amount injected	Day of death postinoculation*	Results of examination of postinoculation sera†: Serum taken	Results of examination of postinoculation sera†: Yellow fever C.F. antigen	Results of examination of liver tissue: Yellow fever C.F. antigen	Pathologic changes‡: Councilman lesions	Pathologic changes‡: Intranuclear inclusions: Classic type	Pathologic changes‡: Intranuclear inclusions: Nicolau type	Pathologic changes‡: Fatty degeneration	Remarks	Histopathologic diagnosis
	mg./kg.		*mg.*		*day*								
Marmoset 14§	100	4	100	8th	6th	none	none	+	+	+	−	Moderate necrosis, many inclusions of both types	Positive for yellow fever
15	100	5	150	5th	3rd, 4th	none	none	+	−	+	+	Moderate necrosis, severe fatty degeneration, congestion	Negative for yellow fever
16	100	5	125	5th	4th	none	none	+	+	+	+	Severe necrosis, intense fatty degeneration, congestion	Positive for yellow fever
17	50	5	47	5th	5th	none	none	+	+	+	+	Moderate necrosis, few inclusions	Positive for yellow fever
18	50	5	40	5th	5th	none	none	+	+	+	+	Moderate necrosis, slight fatty degeneration	Positive for yellow fever
19§	50	6	45	10th	5th, 8th	none	none	+	+	+	+	Moderate necrosis, many inclusions of both types	Positive for yellow fever
20	50	7	105	7th	5th, 6th	none	none	+	−	+	+	Slight necrosis, severe fatty degeneration	Negative for yellow fever
21§	50	8	80	12th	6th, 8th, 10th	none	none	+	+	+	+	Moderate necrosis, congestion	Positive for yellow fver
Monkey 1‖	250	2	2750	14th	3rd, 4th, 5th, 8th, 12th, 14th	none	none	−	+	+	−	Few classic inclusions, many of Nicolau type	Negative for yellow fever
2¶	250	2	2500	12th	4th, 5th, 9th, 12th	none	none	+	−	−	+	Moderate scattered necrosis, intense coagulative necrosis, congestion	Negative for yellow fever

* Number of days after the first injection.
† The symbol "X" indicates serum not tested.
‡ Presence indicated by +, absence by −.
§ Five-day interval between first injection and subsequent daily injections.
‖ Thirteen-day interval between first and second injections.
¶ Ten-day interval between first and second injections.

With one exception (Monkey no. 1), necrosis, although variable in amount and distribution, was seen in the liver of all the animals, regardless of the dosage of tannic acid used. Hyaline necrosis (Councilman lesions) with a scattered, midzonal distribution, a picture regarded as typical of yellow fever, was seen in the livers of all but one of the animals, as shown in table 1. Acidophilic intranuclear inclusions, indistinguishable from those described for yellow fever by Torres (8), and Cowdry and Kitchen (19) were found in the liver of 14 of the animals. The number of inclusions observed in the sections varied considerably between animals, but in the present series of experiments they were found much more regularly in marmosets inoculated with tannic acid than in marmosets succumbing to inoculation of yellow fever virus. Fatty degeneration was present in 19 of the 23 livers; in the pathologic description, the degree in seven was reported as "marked" and in 12 as "moderate."

A description of the microscopic lesions found in the livers of marmosets and monkeys damaged by tannic acid, together with a comparison with the lesions produced in the livers of the same species by yellow fever virus, will be published in detail later (20); from the present brief description, however, it is evident that a basis for confusion in diagnosis exists. The difficulty in distinguishing, at least in marmosets, between damage produced by tannic acid and that produced by the virus is emphasized in table 1: in 13 of the 21 animals, a diagnosis of "positive for yellow fever" was returned by a pathologist with a wide experience in the examination of human and animal yellow fever material.

Nicolau, Kopciowska, and Mathis (4) reported the occurrence in the yellow fever liver of intranuclear inclusions which they believe to represent the virus, and to be different from the classic inclusions, which arise from oxychromatic degeneration of the nucleus. These authors regard the presence of the inclusions described by them as pathognomic of yellow fever; and since, from Belt's publication (12), it was assumed that the lesions caused by tannic acid might in some cases be indistinguishable from those caused by the yellow fever virus, portions of liver were fixed in Duboscq-Brasil, as well as in formalin, and stained by Mann's method, as recommended by the French workers. The Nicolau yellow bodies were found in 16 of the 21 marmoset livers and in one of the two rhesus livers; these bodies, therefore, cannot be considered as specific for yellow fever.

In summary, it was found that in experimental animals the microscopic lesions produced in the liver by tannic acid frequently were indistinguishable from those produced by yellow fever virus and, moreover, that in the animals treated with tannic acid, no antigen capable of fixing complement in the presence of yellow fever immune sera was demonstrable.

The blood of animals infected with the virus, however, contains such an antigen and, as is shown in table 2, the antigen was present even in those cases where the liver lesions were minimal or whose character did not permit of unequivocal diagnosis of yellow fever. The animals listed in table 2 were inoculated with different strains of yellow fever virus and were bled when moribund or when signs of infection had developed. The sera were tested for the presence of virus and of complement-fixing antigen; and the livers, removed at death, were examined for antigen and for the presence of lesions. It will be noted that Councilman lesions were not found in four of the 10 marmosets or in three of the four monkeys examined; the livers of the remaining animals showed varying degrees of necrosis and fatty degeneration. Despite the fact that these animals had been inoculated with virus, which was demonstrable, and usually in high titer, at the time of death, the microscopic diagnosis in four marmosets and two monkeys was returned as "negative for yellow fever" and in three marmosets and two monkeys as "questionable positive"; only three animals of the 14 were diagnosed as positive.

The complement-fixing antigen was readily demonstrable in the blood of all 14 animals, however, so that its demonstration not only served to differentiate yellow fever virus infection from another condition whose pathologic picture in the liver may be indistinguishable from that of yellow fever, but also provided a means of arriving at a diagnosis in cases where microscopic diagnosis was impossible because of the character of the lesions produced by the specific virus.

COMMENT AND SUMMARY

There appears to exist no fixed relationship between the clinical severity of yellow fever virus infection and the amount of damage found in the liver at death (9), and in some, though infrequent, instances, the liver changes in rapidly

TABLE 2

Serologic and histopathologic examination of tissue from animals infected with yellow fever virus

ANIMAL	DAY OF DEATH POST-INOCULATION	RESULTS OF EXAMINATION OF POST-INOCULATION SERUM			RESULTS OF EXAMINATION OF LIVER TISSUE					
						Pathologic changes†				
		Serum taken	Virus	Yellow fever C.F. antigen	Yellow fever C.F. antigen*	Council-man lesions	Intra-nuclear inclusions (classic type)	Fatty de-gener-aion	Remarks	Histopathologic diagnosis
		day								
Marmoset 22	4th	3rd	+	present	present	+	−	−	Moderate necrosis, congestion	Positive for yellow fever ?
23	4th	4th	+	present	none	−	−	−	Slight focal necrosis	Negative for yellow fever
24	5th	4th	+	present	X	+	+	+	Congestion, few necrotic cells, few inclusions	Positive for yellow fever
25	5th	4th	+	present	present	+	+	−	Slight necrosis, few inclu-clusions	Positive for yellow fever
26	6th	5th	+	present	X	+	+	+	Congestion, few necrotic cells, few inclusions	Positive for yellow fever
27	6th	6th	+	present	present	−	−	−	Extensive lymphocytic infiltration	Negative for yellow fever
28	6th	6th	+	present	present	−	−	+	Normal picture	Negative for yellow fever
29	6th	6th	+	present	present	+	−	+	Moderate necrosis, congestion	Positive for yellow fever ?
30	7th	5th	+	present	present	+	−	+	Slight necrosis, severe fatty degeneration, congestion	Positive for yellow fever ?
31	8th	8th	+	present	present	−	−	−	Normal picture	Negative for yellow fever
Monkey 3	5th	5th	+	present	present	+	−	+	Intense necrosis, congestion	Positive for yellow fever ?
4	9th	5th	+	present	none	−	−	+	Severe fatty degeneration	Negative for yellow fever
5	?	?	+	present	none	−	−	+	Congestion, slight necrosis	Negative for yellow fever
6	?	?	+	present	X	−	+	+	Coagulation necrosis atypical of yellow fever present	Positive for yellow fever ?

* X indicates test not done.

† + indicates present; − indicates absent.

? indicates animals under continuous exposure to infection, exact date of infection unknown.

fatal cases are so few as to make diagnosis difficult or impossible. On the other hand, the content of complement-fixing antigen in the blood is roughly parallel to the severity of the infection (1–3) and hence demonstration of the antigen provides a means of diagnosis (2, 21).

The lack of correlation between the antigen content of the serum and the intensity of the lesions in the liver indicates that the antigen does not arise as a result of the visible damage produced. The similarity or practical identity of the lesions produced by the virus and by tannic acid, and the absence of the yellow fever antigen in animals succumbing to the toxic effects of the acid, support this view, and also indicate that the antigen is specific.

Nevertheless, there is some relationship, even though not absolute, between the demonstrable tissue destruction and formation of antigen. Thus, in the monkey and the marmoset, some pathologic change, however minimal, was as a rule observed in the liver, but this was not always true of the other organs. In the mouse, infection by the intraperitoneal route results in the eventual appearance of the antigen in the blood and in equally high or greater concentration in the brain, where the virus exerts its obvious damage, while the liver, which microscopically appears to be unaffected, contains no antigen, or only traces referable to the blood content of the organ (22). Just as the damage provoked in the liver of primates by tannic acid and by the yellow fever virus may be indistinguishable, there is nothing to distinguish the lesions produced by this virus in the brain of the mouse from those caused by other viruses; nevertheless, the antigen produced in the blood of the host infected with the yellow fever virus serves to differentiate this infection from similar conditions of different etiology (3).

The yellow fever complement-fixing antigen, therefore, appears to be biologically specific and the present evidence indicates that its demonstration constitutes a more accurate and reliable method of diagnosis than does histopathologic examination of tissue.

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THE USE OF RHESUS MONKEYS IN THE TESTING OF AQUEOUS-BASE YELLOW FEVER VACCINE

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Living yellow fever virus has been successfully employed since 1931 (1) in the vaccination of man. The object of vaccination is to implant sufficient living virus within the body of the recipient to insure multiplication and subsequent generalized infection. The immunity resulting from such infection is extraordinarily effective (2, 3, 4).

Two attenuated strains of yellow fever virus have been principally employed in vaccination; the French neurotropic strain developed by Theiler (5, 6) and the 17D strain produced by Lloyd, Theiler, and Ricci (7) and Theiler and Smith (8). Both strains have lost the ability to cause visceral yellow fever in either man or rhesus monkey while retaining the power to multiply and to produce immunizing infections. Both possess the capacity to induce yellow fever virus encephalitis in certain susceptible animals following intracerebral inoculation. The one great difference between the two viruses is their degree of neurotropic virulence. Intracerebral injection in adequate dosage of the French neurotropic virus into white Swiss mice or rhesus monkeys produces death in nearly 100 per cent of animals in 4 to 10 days (9, 10). Similar inoculation of the 17D virus into white Swiss mice results in death 7 to 21 days later while like injection of rhesus monkeys is followed only occasionally by a fatal encephalitis (11, 12).

Since 1937, only the 17D strain of yellow fever virus has been employed in the Americas in the vaccination of man. Hargett, Burruss, and Donovan (11) have described the method of vaccine preparation as followed by the United States Public Health Service. This method is a modification of the procedure previously described by Theiler and Smith (9) and Smith, Penna, and Paoliello (13). Considerable care is required in the preparation, testing, and administration of this vaccine on account of its very labile nature (14), alterations in virulence which may occur (12, 15, 16), and the possibility of contamination with pathogenic agents (17, 18). Improperly prepared vaccine or well prepared vaccine which has been improperly stored or abused may contain no active virus or too little active virus to insure immunization (13, 19, 20). Anaphylactic reactions may, rarely, occur in persons sensitive to the chick-embryo-egg proteins contained in the vaccine (21, 22, 23).

The three species of animals employed by the United States Public Health Service in the testing of yellow fever vaccine are white mice of the Swiss strain, guinea pigs, and rhesus monkeys. Mice are used (1) in titrating virus content, (2) in evaluating the degree of neurotropism possessed by the vaccine virus, (3) in detecting circulating virus in the blood stream of test monkeys, and (4) in protection test determinations on pre- and postvaccination monkey serum specimens. Guinea pigs are employed to detect possible virulent contaminants which may fail to grow in culture media. Monkeys (*Macaca mulatta*) are used (1) to evaluate the behavior characteristics of the vaccine virus in producing clinical and pathological signs and symptoms, (2) to determine the ability of the virus to multiply and appear in the blood stream, and (3) to test the power of the vaccine to stimulate specific yellow fever immunity as measured by the protection test. To Stokes, Bauer, and Hudson (24) go the honor of first demonstrating that the rhesus monkey is highly susceptible to yellow fever.

In this paper are reported the observations on a consecutive series of 200 monkeys employed in the testing of *aqueous-base* (serum-free) *yellow fever vaccine* prepared in the laboratories of the National Institute of Health.

VACCINE AND ANIMALS

Vaccine. One hundred and ninety-two different lots of vaccine were employed in this study. These were taken from a consecutively prepared series of 193 lots, one of which was excluded be-

cause of a suspected bacterial contamination. Each lot was prepared essentially as follows:

Seven day old chick-embryos were inoculated with seed virus, incubated an additional 3 to 4 days, removed from the shells, and triturated in blendors after adding *1.00 ml. of distilled* water per *3.00* gm. of embryo. The resulting emulsion was centrifuged and the supernatant drawn off into storage bottles in which it was shell-frozen and temporarily stored at minus 78°C. pending determinations of contamination and virus content. Bottles of frozen supernatant found to be free of contaminants and of adequate virus content were then melted at 37°C. and distributed into ampoules. The distributed vaccine was then promptly shell-frozen and subsequently dried from the frozen state under high vacuum. The ampoules of desiccated vaccine were then filled with dry nitrogen, sealed, inspected, labeled, stored at minus 15°C. to minus 30°C., and tested as to suitability for use. Neither human nor animal serums were employed in the preparation of these lots (11).

The original 17D yellow fever virus used in preparing these vaccines was kindly supplied by J. H. Bauer of the Rockefeller Foundation. All vaccines were made with seed virus which had undergone 230 to 233 previous tissue-culture or egg-culture passages. For identification purposes *this virus has been designated substrain 17D-RML.*

The concentration of virus in the vaccines, as determined by the 50 per cent endpoint titration method of Reed and Muench (25), varied from 140 to 1,250,000 with a median value of 67,450. Titrations were performed from 1 to 17 days prior or subsequent to inoculation of the monkeys. Decimal dilutions were employed. With one exception in which 24 mice were used, 6 or 12 animals were injected with each dilution. The titer values and the m.l.d.[1] determined therefrom are to be regarded as approximate only. On the basis of m.l.d. of vaccine virus received per animal, the 200 monkeys studied have been divided, as shown in table 1, into 2 groups as follows: a "low virus" group of 102 animals in which each individual received 200 to 199,000 m.l.d. and a "high virus" group of 98 animals in which each individual received 202,000 to 2,230,000 m.l.d. Unfortunately, 2 groups of 100 each could not be established without including 1 or more *animals in each group which had received* an identical number of m.l.d. of virus. Most of the tables presented have been constructed to show results for each of these groups and for the two combined.

In preparing the vaccines for inoculation, distilled water was employed for rehydration and 0.85 per cent sodium chloride solution for dilution.

Monkeys. The 200 *rhesus monkeys used in* this study were within a weight range of 5½ to 12 pounds. One hundred and seventy-eight of these animals came directly from commercial dealers and had not previously been subjected to experimentation. Twenty-two came from other laboratories and had been previously used in studies of infectious diseases as follows:

14 Poliomyelitis
5 Mumps and measles
2 Mumps
1 Rocky Mountain spotted fever[2]

Prior to use, the animals were kept well removed from the yellow fever laboratory. They were housed in large cages under excellent hygienic conditions and supplied an adequate diet of high quality. Some weeks prior to injection with vaccine, each animal was bled from a leg and the blood tested by the virus-neutralization test for evidence of immunity to yellow fever. Only those monkeys demonstrated to be non-immune to this disease and in robust health were employed in the testing of vaccine.

Mice. All mice were of the white Swiss strain raised from a single inbred colony at this laboratory. The animals were between 29 and 60 days of age, except those employed in the young-mouse protection test, and apparently in good health at the time placed in use. None had been subjected to prior experimentation.

METHODS AND STANDARDS

Intercerebral inoculation of monkeys. The hair was clipped, and in some instances shaved, from that area above the right brow and the clipped skin washed with soap and water. The animal was then placed under light ether anesthesia and the just-washed skin painted with tincture of iodine. The point of injection was then located; this was approximately 1 inch above the middle of the right superior orbital ridge. The scalp over this point was then opened by an incision of about ½ inch *in length. A small hole just large enough to ad-*

[1] Refer to subsequent section "Methods and Standards."

[2] It has not been possible to determine for a certainty whether or not the last monkey listed had actually been used in spotted fever studies. In this paper it is considered to have been so employed.

mit a 25-gauge hypodermic needle was then made through the skull. Employing a 1.00 ml. tuberculin syringe fitted with a ½ inch 25-gauge needle, the vaccine was injected slowly into the right frontal lobe of the brain with care to avoid unnecessary trauma. Following inoculation, the skin at site of incision was brought together and the wound covered with collodion.

Drawing monkey blood specimens. All specimens were taken from the leg vessels. Five to 10 ml. were drawn for virus-neutralization test determinations and 2 ml. for detection of circulating virus.

Fever of monkeys. A rectal temperature of 40.0°C. or higher was considered as an indication of fever. Whenever such a temperature was encountered it was promptly confirmed employing a different thermometer. In those instances where multiple fever readings were recorded for a single day, only the highest has been included in the data presented.

Circulating virus in monkeys. The method described by Theiler and Smith (8) has been employed to detect the presence of yellow fever virus in the blood stream. Promptly following withdrawal of 2 ml. of blood the serum was separated and 0.03 ml. inoculated intracerebrally into each of a group of 6 mice. The mice were then observed for 21 days and deaths and survivals recorded. Animals dying from causes apparently other than yellow fever virus encephalitis were eliminated from consideration. The death of 1 or more mice of a given group from *apparent* yellow fever virus encephalitis was regarded *as evidence of* circulating virus.

Yellow fever virus-neutralization tests. Monkey serums were examined for yellow fever virus-neutralizing bodies by the intraperitoneal method of Sawyer and Lloyd (26) employing 3 ml. of a 1:3 dilution of serum in 0.85 per cent sodium chloride solution. Two of the serums were also examined by the young-mouse method described by Whitman (27). Results were interpreted according to the standards set forth by Sawyer (28).

"Slight to moderate weakness" of monkeys. This was regarded as a loss of strength varying from the least detectable by handling and observing the animal to the most marked degree which still permitted the subject to stand.

"Severe weakness" of monkeys. This was considered as asthenia of such a degree that the monkey was unable to stand.

Encephalitis of monkeys. Diagnosis was based on the 3 criteria—weakness, paralysis, and pathology of the brain. Histopathological examination of the brains of those animals coming to autopsy was kindly made by R. D. Lillie, Chief, Pathology Laboratory, National Institute of Health.

Autopsy of monkeys. This was undertaken promptly after death. The contents of the abdominal and thoracic cavities were inspected and the brains removed and fixed.

Period of observation of animals. This commenced with the day of inoculation and continued to day of death or terminus of study period. With mice used for virus titration or for the detection of circulating virus the period was always 21 days unless shortened by death. With monkeys it was 26 to 31 days unless shortened by death or extended for special reason.

Minimum lethal mouse dose (m.l.d.). Mice of a susceptible strain, as the white Swiss, inoculated intracerebrally with yellow fever virus usually die within three weeks of yellow fever virus encephalitis. One m.l.d. of virus is that quantity which injected intracerebrally into each of a series of such mice is followed by the death of one-half of them from encephalitis (25).

PROCEDURE AND RESULTS

Inoculation. Monkeys selected to receive vaccine were first brought into the yellow fever laboratory on the day of inoculation. Just prior to injection of vaccine a blood specimen was taken for protection test study. The serum was separated and temporarily stored at 3° C. to 6° C. The animals were then injected intracerebrally with vaccine. The volume of inoculum was in every case 0.25 ml. while the dilution ranged from 1:1 to 1:10. The m.l.d. received per monkey varied from 200 to 2,230,000 with a median of 193,500 as shown in table 1. In one instance vaccine from a single lot was injected into 3 monkeys while in 6 instances vaccine from single lots was injected into 2 monkeys. One hundred and eighty-five additional lots were injected into a like number of monkeys. No animal received vaccine of more than 1 lot.

Observation. Following inoculation each monkey was housed in a separate cage under excellent hygienic conditions and fed a liberal diet of high quality. Seven animals were observed for 11 to 24 days, 183 for 26 to 31 days, and 10 for 32 to 60 days. All 7 of the first group became profoundly ill and died or were sacrificed. Observation was

extended with the third group, usually on account of persistent weakness or paralysis.

Circulating virus. Each monkey was bled on the 2nd, 3rd, and 4th postinoculation days for the detection of yellow fever virus in the blood stream as a previous study (29) with serum-base vaccines indicated that virus was most likely to be encountered during this period. This is in agreement with similar observations reported by Fox and Penna (12). Eight monkeys (4 per cent) showed no evidence of circulating virus. Serums from 4 animals (2 per cent) killed all mice (a total of 72) with apparent yellow fever virus encephalitis. Widely divergent results between the two extremes just mentioned were encountered with the 188 remaining monkeys. A summary of results is shown in table 2. It is to be noted that the mortality of mice inoculated with serums from the "high virus" group of monkeys was greater than those inoculated with serums from the "low virus" group.

Febrile temperatures. The rectal temperature of each monkey was determined daily throughout the period of observation. Thirty-three animals (16½ per cent) showed no fever at any time. The remainder had temperatures varying from 40°C. to 41.0°C. and continuing for 1 to 7 days. Median elevation was 40.2°C. and median duration 2 days. The average febrile duration was slightly less than 2 days. The great majority of febrile temperatures were registered on the 8th, 9th, and 10th postinoculation days. Results are embodied in tables 3 and 4.

Virus-neutralization tests. Postvaccination blood specimens for detection of yellow fever virus-neutralizing bodies were taken 12 to 33 days subsequent to injection of monkeys with vaccine except from 1 animal (no. 329) which died on the 11th postinoculation day. The specimens were usually taken on the 14th postinoculation day as it is known (8) that rhesus monkeys injected with an adequate dose of 17D virus develop neutralizing bodies within 2 weeks. The drawing of some specimens was delayed on account of weakness of certain animals on the 14th day.

Those monkey serums obtained just prior to

TABLE 1

The number of m.l.d. of vaccine virus received by the 200 test monkeys

Inoculated intracerebrally	M.L.D. of virus per monkey													Total
	200–99,999	100,000–199,000*	202,000–299,999	300,000–399,999	400,000–499,999	500,000–599,999	600,000–699,999	700,000–799,999	800,000–899,999	900,000–999,999	1,000,000–1,499,999	1,500,000–1,999,999	2,000,000–2,230,000	
No. of monkeys														
"Low virus"	72	30												102
"High virus"			20	12	11	9	8	12	7	3	12	2	2	98

* Median number of m.l.d. of virus is 193,500.

TABLE 2

Yellow fever virus in the blood stream of test monkeys following intracerebral injection of yellow fever vaccine as indicated by inoculation of susceptible mice

Monkeys		Yellow fever virus in the blood stream					
		2nd day post-inoculation		3rd day post-inoculation		4th day post-inoculation	
M.l.d. of virus per animal	Number observed	Mortality* ratio	Per cent mortality	Mortality ratio	Per cent mortality	Mortality ratio	Per cent mortality
200 to 199,000 ("low virus")	102	266/601	44	340/600	57	323/604	53
202,000 to 2,230,000 ("high virus")	98	302/581	52	345/582	59	350/571	61
Total	200	568/1182	48	685/1182	58	673/1175	57

* Denominator indicates number of mice under test and numerator the number dying during the 21 days subsequent to injection.

the injection of vaccine, which had been held in cold storage, were matched with the postinoculation serums from the same animals. Each pair of serums was placed in a single protection test "run." The protection test results of the pre- and postinoculation serums were thus strictly comparable. Fortunately none of the specimens were contaminated and all protection test "runs" proved satisfactory. All preinoculation serums showed yellow fever virus-neutralizing bodies absent and all postinoculation specimens showed them present. Two of the latter gave inconclusive results by the intraperitoneal method of Sawyer and Lloyd (26). Reexamination by the more sensitive young-mouse method of Whitman (27), however, showed the bodies to be present in both specimens. Results are shown in table 5.

Encephalitis. The presence and degree of weakness and paralysis was ascertained by daily observation and handling, principally at the time the monkeys were removed from their cages for temperature determinations. Fifty-four (27 per cent) showed weakness varying from the least detectable to that associated with the moribund state, while 146 (73 per cent) showed no evidence of loss of strength. Twenty-two of the 54 came from the "low virus" group of 102 monkeys which had received 200 to 199,000 m.l.d. of virus while the other 32 came from the "high virus" group of 98 which had received 202,000 to 2,230,000 m.l.d. Onset of weakness varied from the 9th to the 26th postinoculation day with 50 of the 54 animals first showing weakness on the 10th to 14th days. Median day of onset was the 12th. Forty-two of these 54 animals showed only slight to moderate weakness without evidence of paralysis and 12 developed severe weakness and/or paralysis as shown in table 6.

TABLE 3

The febrile temperatures observed in test monkeys are arranged to show on which post-inoculation day they occurred

Monkeys		Days subsequent to injection of vaccine																	
		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	29	
M.l.d. of virus per animal	Number observed	Number of animals which showed fever on each post-inoculation day																	Total
200 to 199,000 ("low virus")	102	0	1	0	1	1	5	35	57*	41	10	4	3	1	2	1	1	0	163
202,000 to 2,230,000 ("high virus")	98	1	0	0	1	2	8	39	59*	37	12	2	1	1	0	0	0	1	164
Total	200	1	1	0	2	3	13	74	116*	78	22	6	4	2	2	1	1	1	327

* Median.

TABLE 4

The number of test monkeys which showed fever (40°C. and higher) and which failed to show fever during the observation period subsequent to vaccine injection. The animals in the febrile group are listed to show the number of days duration of fever

Monkeys		Number of animals which failed to show fever	Number of animals which showed fever according to number of days febrile						
M.l.d. of virus per animal	Number observed		1 day	2 days	3 days	4 days	5 days	6 days	7 days
200 to 199,000 ("low virus")	102	20	32	30*	15	2	1	1	1
202,000 to 2,230,000 ("high virus")	98	13	33	32*	15	3	2	0	0
Total	200	33	65	62*	30	5	3	1	1

* Median.

TABLE 5

Protection test mortality ratios obtained with monkey serums taken just prior to injection of vaccine and with serums taken from the same animals 12 to 33 days subsequent to vaccine inoculation.

Pre-inoculation monkey serum specimens

DAYS BEFORE VACCINE INJECTION	NUMBER OF SPECIMENS	PROTECTION TEST MORTALITY RATIOS (NUMBER OF MICE DYING/NUMBER OF MICE UNDER TEST)				
		6/6	5/6	4/6	5/5	4/5
0	199	109	67	17	5	1
Total number of specimens 199		Protection test composite result 1086/1188			Per cent of mice which died 91.4	

Post-inoculation monkey serum specimens

DAYS AFTER VACCINE INJECTION	NUMBER OF SPECIMENS	PROTECTION TEST MORTALITY RATIOS (NUMBER OF MICE DYING/NUMBER OF MICE UNDER TEST)				
		0/6	1/6	2/6	3/6	0/5
12	1	1				
13	3	1	2			
14	176	148	24	1*	1*	2
15	4	2	1			1
16	4	3				1
17	2	2				
21	4	4				
22	1	1				
23	1	1				
25	1	1†				
28	1	1				
33	1	1				
Total number of specimens 199		Protection test composite result 32/1190			Per cent of mice which died 2.7	

* A result of 0/6 was obtained when this specimen was retested by the young mouse method of Whitman.

† A result of 4/6 was obtained with serum taken from the same monkey on the 14th post-inoculation day.

The occurrence of fever and circulating virus in relation to the development or non-development of weakness and paralysis was as follows:

The 146 monkeys which did not develop weakness or paralysis:

125 (86 per cent) showed fever.

141 (97 per cent) showed evidence of circulating virus.

The 54 monkeys which developed weakness or paralysis:

42 (78 per cent) showed fever.

51 (94 per cent) showed evidence of circulating virus.

All 42 members of the group which showed slight to moderate weakness without paralysis made what was regarded as a complete recovery during the period of observation. Duration of weakness varied from 1 to 31 days with a median length of 8 days. Ten of this group were weak for 1 to 5 days, 22 for 6 to 14 days, and 10 for 15 to 31 days. The terminal postinoculation day of observed weakness varied from the 12th to the 42nd with the median being the 20th.

Ten of the 12 monkeys which developed severe weakness and/or paralysis died or were sacrificed, 1 made a complete recovery from an apparently moribund state without evidence of paralysis at any time, and the remaining animal recovered except for a residual paralysis of the right hind leg. Brief abstracts from the records of these monkeys follow ("day" refers to postinoculation day):

No. 112. ....137,800 m.l.d. of virus were injected. Fever of 40.0°C. occurred on the 9th day. Weakness was evident from the 11th to the 42nd day. The animal was almost moribund on the 13th day. Clinical recovery was complete on the 43rd day.

No. 132... . 95,000 m.l.d. of virus were injected. Fever of 40.0°C. to 40.5°C. occurred on the 9th and 10th days. Paralysis of the right hind leg developed on the 25th day (or 20th day—the record is defective) and persisted at termination of observation on the 40th day at which time the animal appeared to otherwise be in good health.

No. 141.....52,100 m.l.d. of virus were injected. Fever of 40.5°C. was recorded on the 8th day. Weakness was present from the 10th day until death. Paralysis of the left front and hind legs developed on the 18th day and persisted until death. The animal was sacrificed on the 28th day in a moribund condition. An attempt to recover yellow fever virus from the brain failed. The brain showed encephalitis.

No. 185..... 144,500 m.l.d. of virus were injected. Fever of 40.2°C. was encountered on the 10th day. Weakness was evident from the 11th day until death. Paralysis of both hind legs developed on the 21st day and persisted until death. The monkey was sacrificed on the 24th

day in a weak but not moribund condition. The liver showed probable tuberculosis and the brain encephalitis.

No. 202.....222,000 m.l.d. of virus were injected. Fever of 40.0°C. was observed on the 10th day. Weakness was present from the 12th day until death. The animal was sacrificed on the 33rd day in a moribund condition. The brain showed encephalitis.

No. 230.....951,000 m.l.d. of virus were injected. Fever was not observed. Weakness was present from the 10th day until death. The monkey was sacrificed on the 14th day in a practically moribund state. The brain showed polioencephalitis.

found in the upper lobe of the left lung. The brain showed encephalitis.

No. 280.....772,000 m.l.d. of virus were injected. Fever of 40.0°C. was encountered on the 8th and 9th days. Weakness developed on the 11th day and continued until death. The animal was sacrificed on the 12th day in a very weak condition. A considerable number of adhesions were present between the upper right and upper left lobes of the lungs and the chest wall. The brain showed encephalitis.

No. 308.....416,600 m.l.d. of virus were injected. Fever of 40.2°C. was registered on the 9th day. Weakness developed on the 12th day and continued until death.

TABLE 6

Monkeys which developed weakness and paralysis following intracerebral injection of yellow fever vaccine

MONKEYS		WEAKNESS AND PARALYSIS				
		Slight to moderate weakness (able to stand)		Severe weakness (unable to stand)		Total
M.l.d. of virus per animal	Number observed	Paralysis not observed	Paralysis present	Paralysis not observed	Paralysis present	
200 to 199,000 ("low virus")	102	18	1	1	2*	22
202,000 to 2,230,000 ("high virus")	98	24	2*	4*	2*	32
Total	200	42†	3	5	4	54

* Died or sacrificed.

† Completely recovered during post-vaccination observation period.

No. 242.....738,000 m.l.d. of virus were injected. Fever of 40.0°C. to 40.4°C. was observed on the 9th and 10th days. Weakness was present from the 12th day until death. Paralysis of both hind legs was present from the 15th day until death. The animal was sacrificed on the 17th day in a very weak condition. The brain showed encephalitis.

No. 244.....501,000 m.l.d. of virus were injected. Fever of 40.0°C. to 40.3°C. was registered on the 8th and 9th days. Weakness appeared on the 10th day and continued to death. Paralysis of the right front leg developed on the 11th day and continued to death. The monkey was sacrificed on the 14th day in a practically moribund state. A hemorrhage 5 mm. in diameter was

Paralysis of both hind legs was apparent on the 13th day and continued to death. This animal was kept under observation until the 60th day at which time it was sacrificed. Vital functions seemed to be good at time of death and it is believed this monkey could have survived considerably longer with special care. The brain showed slight residual encephalitis.

No. 327.....337,500 m.l.d. of virus were injected. Fever was not encountered. Weakness developed on the 10th day and continued until death. Paralysis of the left hind leg developed on the 13th day and persisted to death. The monkey was sacrificed on the 39th day at which time the animal was in fairly good general health, although weak.

The brain showed diffuse fairly active widespread encephalitis.

No. 329.....488,000 m.l.d. of virus were injected. Fever was not observed. Weakness developed on the 10th day and continued until death. The animal died on the 11th day. At autopsy a well developed fetus was found and a considerable number of adhesions encountered between the lower left pulmonary lobe and the chest wall; the large intestine was hemorrhagic. The brain showed general encephalitis.

The basic pathologic alterations in the brains examined resembled in general those described by Lloyd and Penna (10) in their studies on the action of the French neurotropic strain of yellow fever virus in rhesus monkeys.

Reactions of different monkeys to identical vaccines. The reactions of the 15 monkeys which received vaccine from 7 different lots permit some observations on the behavior of identical material in different monkeys. There was no consistency of reaction; the fact that 1 animal showed fever, circulating virus, or weakness was no indication

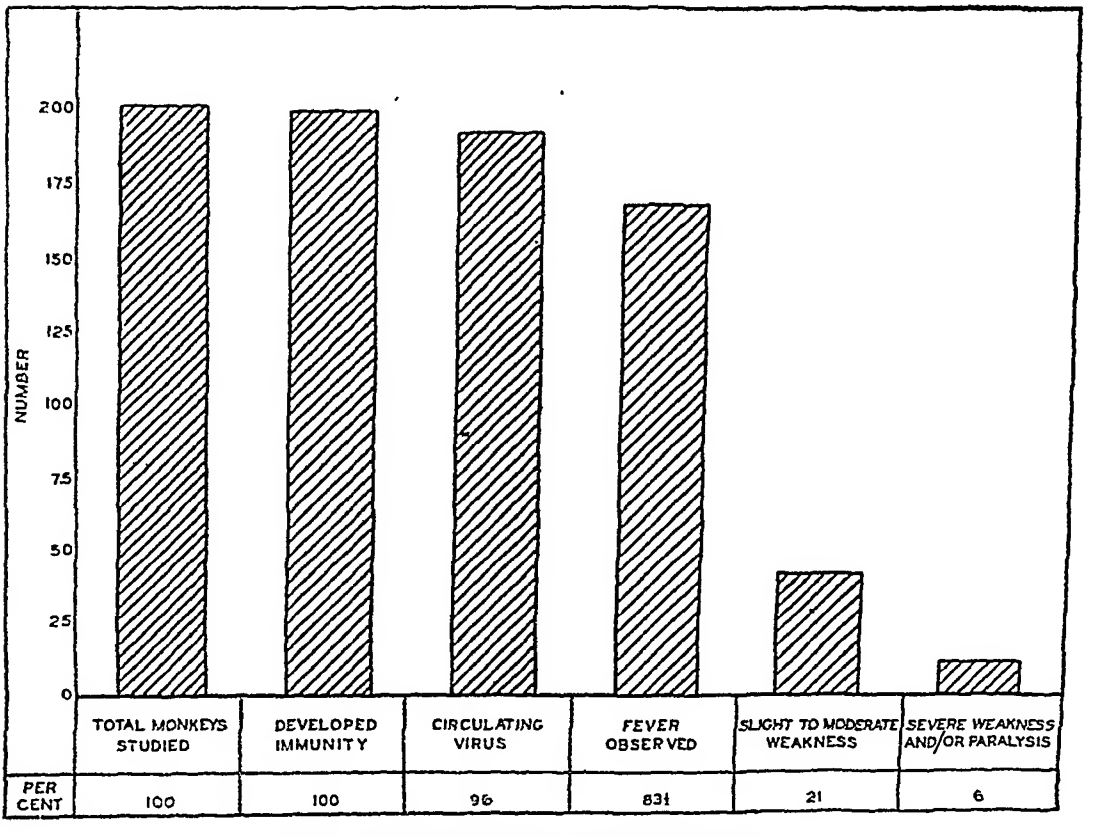

FIG. 1. REACTIONS OBSERVED IN TEST MONKEYS

It is to be noted: (1) that none of these 12 monkeys showed abnormalities during the first week subsequent to injection of the vaccine, (2) that the initial appearance fever was always on the 8th, 9th, or 10th postinoculation day, (3) that the onset of weakness was in every case on the 10th, 11th, or 12th day following injection, (4) that paralysis first appeared on the 11th to 25th postvaccination day, and (5) that encephalitis was encountered in the brains of the 10 animals that came to autopsy.

that another animal inoculated with the same vaccine would react in like manner.

Reactions of monkeys previously employed in infectious disease studies. Of the 22 monkeys which had been previously employed in measles, mumps, poliomyelitis, or Rocky Mountain spotted fever studies, 8 (36 per cent) developed slight to moderate weakness without evidence of paralysis with complete recovery. None of these animals showed severe weakness or paralysis and none died or was sacrificed. In comparison, 46 (26 per cent) of

the 178 monkeys which had not previously been used showed weakness or paralysis or both; all 12 animals which developed severe weakness and/or paralysis were in this group.

DISCUSSION

Intercurrent disease. No monkey of this series died of intercurrent infection. This is attributed to the selection of the animals and the care given them.

Inoculation trauma. The small volume of 0.25 ml. of inoculum was employed in the intracerebral inoculation of monkeys in order to reduce the possibility of trauma inherent in the use of larger volumes. It is not believed that the injury caused by injection played any appreciable part in the reactions observed. None of the inoculation wounds became infected and on inspection at autopsy none of the brains showed evidence of trauma. That injury caused by intracerebral injection may cause death seems indicated by a case reported by Lloyd and Penna (10): a monkey known to be immune to yellow fever was injected intracerebrally with yellow fever virus, the animal died, and at autopsy an area of softening and hemorrhage was encountered in the lobe which had received the virus.

Yellow fever virus content of inoculums. Dilution of the vaccines inoculated into monkeys was usually employed to reduce the number of m.l.d. of virus received by the animals. As shown in table 1, the smallest number given a monkey was 200 and the greatest 2,230,000. This is considerably more than the minimal infective dose which has been shown (9) to be but a fraction of 1 m.l.d.

The results presented in table 6 indicate that frank encephalitis is more likely to occur when a large amount of virus is injected rather than when a smaller amount is given. In the present study, of the 18 monkeys which received less than 22,000 m.l.d. only 1 showed weakness while of the 16 which received more than 1,000,000 m.l.d., 5 developed weakness. All 6 animals completely recovered during the period of observation. Information does not permit a statement as to the optimal number of m.l.d. that should be employed. Certainly the number should be sufficiently large to insure dependability of the test and yet not so great as to induce a high per cent of cases of severe encephalitis.

Circulating virus. As to the 8 monkeys in which no evidence of circulating virus was obtained, it is to be remembered that search for it was made only 3 times per animal. If more frequent bleedings over a longer period had been attempted, evidence might have been encountered. That all 8 animals became infected is indicated by the demonstration of yellow fever virus-neutralizing bodies in their postinoculation blood specimens. Five of the 8 showed fever and 3 developed weakness. One (no. 308) was sacrificed, and examination of the brain revealed encephalitis. Three showed neither fever, weakness, nor paralysis. The amount of virus received by these 8 monkeys ranged from 180,600 to 501,000 m.l.d.

The results shown in table 2 indicate what is believed to be a significantly greater mortality among mice which received serum from monkeys injected with more than 200,000 m.l.d. as contrasted with those which received serum from monkeys injected with less than 200,000 m.l.d. This result is in agreement with data presented by Fox and Penna (12).

Febrile temperatures. Results listed in tables 3 and 4 show that with the "low virus" group of monkeys as compared with the "high virus" group (1) the likelihood of fever developing is slightly less, (2) the average interval between injection of vaccine and appearance of fever is just a little greater, and (3) the duration of fever is slightly shorter. Whether or not these slight differences are significant may be questioned. The frequency with which febrile temperatures occurred on the 8th, 9th, and 10th postinoculation days and the duration of the fever for 1, 2, or 3 days is noteworthy. On the basis of Fox and Penna's (12) contention that length of fever in rhesus monkeys inoculated intracerebrally with 17D virus is an indication of the neurotropic virulence of the virus, then the virus injected into these 200 monkeys must be considered as possessed with unusually low neurotropism as the median duration of fever in the 167 animals which showed fever was only 2 days and the average slightly less.

Yellow fever virus-neutralizing bodies. Every one of the 199 monkeys studied developed yellow fever virus-neutralizing bodies subsequent to the injection of vaccine. This indicates that the inoculum in each instance contained an infective dose of yellow fever virus and that each of the animals was competent to respond with the production of immune bodies. As it was shown (30, 31) that the concentration of protective bodies developing subsequent to injection of yellow

fever virus bore no relation to the size of the infecting dose, there was no occasion to undertake a study of the concentration of these bodies in the postinoculation serum specimens.

Encephalitis. That rhesus monkeys inoculated intracerebrally with more than 200,000 m.l.d. of 17D virus are more likely to develop signs of encephalitis than animals receiving less than 200,000 m.l.d. is indicated by the results shown in table 6. Six per cent of all monkeys developed severe weakness and/or paralysis. Ten of the 12 animals which composed this group died or were sacrificed and encephalitis proved in each case by pathological study. Fox and Penna (12) working with both serum-containing and serum-free 17D vaccines found that 5.9 per cent of their monkeys developed "marked" or fatal encephalitis subsequent to intracerebral inoculation of vaccine. However, they failed to note any significant difference in the incidence of encephalitis related to virus dose.

It is evident, as has been pointed out before (11, 12), that 17D virus possesses a degree of neurotropic virulence sufficient, when inoculated intracerebrally, to cause death in a small per cent of rhesus monkeys. In agreement with all previously reported experience with this virus in these monkeys, no evidence of visceral lesions of yellow fever was encountered.

Variations in monkey susceptibility. It has been pointed out by Davis (32) that inherent constitutional factors are of considerable influence in relation to reactions developing subsequent to injection of rhesus monkeys with yellow fever virus. In the series of monkeys here reported there was no more similarity of reaction among those which had been injected with vaccine from a single lot than among those injected with material from different lots. It is believed that the 2 most important factors relative to the development of encephalitis in these monkeys were the inherent susceptibility of the individual animal and the amount of virus injected.

Monkeys employed in prior studies of infectious diseases. The differences in behavior of the 22 monkeys of this series which had been previously used in infectious disease studies compared with the 178 previously unused animals are not regarded as significant. It is to be remembered, however, that every one of these 22 animals was in apparent robust health at the time injected with vaccine. As prior use introduces an added variable factor, it is preferable to employ only fresh stock animals.

Etiology of observed reactions. On the basis of evidence presented in this report it is believed that the reactions observed in the 200 monkeys studied resulted from the 17D virus injected into the brains of these animals. This belief is supported by the general similarity of these observations to those reported by Fox and Penna (12) in a comparable study. While it would be difficult to prove that none of the inoculums contained a contaminant, there is no proof that any did.

Vaccine in man. Of the 192 lots of vaccine which constitute this series, 174 have been released for human use, principally to the United States Army. Every one of a total of 177 postvaccination serum specimens procured from persons vaccinated with one or another of 25 of these lots was found to contain yellow fever virus-neutralizing bodies. No serums representing the remaining 149 lots have to date been obtained.

Postvaccination hepatitis reactions, such as occurred in the United States Army in 1942, have been shown to be associated with vaccine lots prepared with human serum (17, 18, 33). As these 192 lots of *aqueous-base vaccine* contained no human (or animal) serum, it was not anticipated that such reactions would occur. Such has proved to be the case; in fact, no untoward reactions of any kind that could be attributed to these vaccines have been reported.

17D virus for human immunization. Despite the fact that white Swiss mice almost always die and rhesus monkeys occasionally die of yellow fever virus encephalitis following the intracerebral inoculation of 17D virus, and that human cases of encephalitis following vaccination with 17D virus have been reported (16), it is still believed that *serum-free 17D virus vaccine* is a safe agent for the immunization of man against yellow fever provided proper care is taken in the preparation, testing, storage, and administration of the vaccine.

SUMMARY

One hundred and ninety-two lots of *aqueous-base* (serum-free) *yellow fever vaccine* were tested in 200 rhesus monkeys (1) to evaluate the behavior characteristics of the vaccine virus in producing clinical and pathological signs and symptoms, (2) to determine the ability of the vaccine virus to multiply and appear in the blood stream, and (3) to test the power of the vaccine to stimulate specific yellow fever immunity. The monkeys

constitute a strictly consecutive series. All lots of vaccine employed likewise were of a consecutive series broken only by the absence of 1 lot which was not tested in a monkey because of possible bacterial contamination.

Each monkey was inoculated intracerebrally with 0.25 ml. of yellow fever vaccine from a single homogeneous lot. The inoculums contained from 200 to 2,230,000 minimum lethal mouse doses (m.l.d.) of virus each. The animals were observed for a median period of 30 days subsequent to inoculation. For comparative purposes the 200 monkeys were divided into two groups, viz., a "low virus" group of 102 animals, each of which received 200 to 199,000 m.l.d. and a "high virus" group of 98, each of which received 202,000 to 2,230,000 m.l.d.

No monkey died of trauma or from intercurrent disease.

Each monkey was bled on the 2nd, 3rd, and 4th postinoculation days to determine if vaccine virus was present in the circulation. Evidence of its presence was found in 192 animals (96 per cent). Results indicated that during these days more virus was present in the blood of the "high virus" group of monkeys than in the "low virus" group.

Daily rectal temperature determinations were taken throughout the postinoculation observation period. A temperature of 40°C. or higher was regarded as fever. One hundred and sixty-seven of the monkeys (83½ per cent) showed fever. The very great majority of febrile temperatures occurred on the 8th, 9th, and 10th days subsequent to the injection of vaccine. Median duration of fever was 2 days.

The pre- and postinoculation yellow fever immunity status of 199 of the 200 monkeys was studied by the protection test. All 199 animals proved to be nonimmune immediately prior to vaccination and all proved to be immune 12 to 33 days later.

Of the 102 animals in the "low virus" group, each of which received 200 to 199,000 m.l.d. of virus, 18 (17.6 per cent) showed transient weakness with complete recovery and 4 (3.9 per cent) developed signs of severe encephalitis characterized by extreme weakness and/or paralysis. Of these 4, 2 were sacrificed, 1 recovered, and 1 was left with a residual paralysis of the right hind leg. Of the 98 monkeys in the "high virus" group, each of which received 202,000 to 2,230,000 m.l.d. of virus, 24 (24.5 per cent) showed transient weakness with complete recovery and 8 (8.2 per cent) developed signs of severe encephalitis. One of these 8 died and the other 7 were sacrificed. Histopathological study of the brains of the 10 animals coming to autopsy showed encephalitis in each instance.

The 2 most important factors in producing encephalitis in these monkeys are believed to be the inherent susceptibility of the individual animal and the amount of virus injected.

Twenty-two of the monkeys studied had been previously used in studies of poliomyelitis, mumps, measles, or Rocky Mountain spotted fever while 178 had not been previously subjected to experimentation. No significant differences in reaction were observed between the 2 groups.

Of the 192 vaccines studied, 174 were released for human vaccination, principally to the United States Army. No icterogenic or other untoward reactions indicating faulty vaccine have been encountered.

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SEROLOGICAL AND ENTOMOLOGICAL SURVEY OF MURINE TYPHUS

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The current problem of endemic typhus in the South and South-east necessitates more determined action towards the eradication of the reservoir rodent host, primarily from the infective foci. The conventional technique for detecting the disease in rodent populations involves the inoculation of rat brain or insect emulsions into guinea pigs and then the checking of the temperature reactions for the succeeding two or three weeks. If a thermal reaction is induced the problem is then resolved into identification by serum protection or immunization experiments. This entire process generally requires a minimum of three weeks, necessitating considerable assistance and materials.

During the past seven months the Eighth Service Command Laboratory has been interested in the typhus problem in and around San Antonio. Rats were originally examined by the guinea pig inoculation technique referred to above; however, it was found that the combined serological and entomological examination of the rats would give results of comparable significance. This was finally evolved into the following approach:

Rats were trapped and delivered to the laboratory in the living state. They were placed in insect proof sacs and anaesthetized with ether. When unconscious the rats were bled from the heart with a 5 cc. syringe and 20 gauge needle. The serum was then separated from the contracted clot and inactivated at 61°C. for one-half hour. Heat coagulation was avoided by diluting this serum two-fold with normal saline. The conventional complement fixation test was set up with 1:10 dilution of serum, two units of rickettsial antigen, and two units of complement. Following an incubation period of forty-five (45) minutes at 37°C. the amboceptor and red blood cells were added and further incubated for thirty (30) minutes.

The exsanguinated rat and the everted sac in which it was anesthetized were spread out on a clean white cloth under a strong light. The ectoparasites were transferred to a small dish of 70% ethyl alcohol with dissecting needles or small sharp pointed forceps. Identification of these arthropods was best completed with a dissecting microscope, or, after some experience, with a good hand lens.

Of the seventy-five rats (*Rattus alexandrinus*) examined from suspected foci of infection, twenty-five (33.3%) were positive serologically for endemic typhus. These included six of thirty-four (17.6%) from theatres, and sixteen of thirty (53%) from food establishments. Ectoparasites were found on all of the positive cases and on some of the negatives: these consisted of three hundred and fifty-seven (357) tropical rat mites (*Liponyssus bacoti*) and forty-four (44) rat fleas (*Xenopsylla cheopis*). Five additional rat serums were anticomplementary.[2]

Seventy-five control rats which originated from non-suspected foci were similarly collected and examined. None was positive serologically. Three fleas and four mites were collected from this group.

In order to determine the experimental serological reaction of rats to typhus, twenty-five white rats were inoculated intra-peritoneally with 1.0 cc. of an emulsion of typhus infected guinea pig spleen. Two rats from this group were checked at weekly intervals for complement-fixing antibodies. One of the two rats which were examined after one week was positive; one week later both of the rats which were tested were positive. To date, the reaction has been uniformly positive at 120 days after inoculation in all of the rats tested.

The rationale of substituting the specific (1) complement fixation test for the actual isolation of the agent is justified by the results obtained. It is known that the typhus agent can be isolated from the blood of the rat as long as two to four weeks after exposure and that thereafter it becomes non-infective (2). Furthermore, the typhus agent has been isolated from the brain of the rat as long as three hundred and seventy (370) days after

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[2] More recently, 195 rats from food establishments were examined, and 55.3% were found to be positive for typhus.

inoculation (3). It is reported that once the flea becomes infected it remains infective for the rest of its life (4). Therefore, a successful isolation from the brain does not necessarily connote an ability to transfer the disease to a vector. However, if known vectors of typhus such as the flea and mite are found on positive cases it is reasonable to consider the insects as potentially infected. The serology will remain positive and specific at least as long as the agent is residing in the brain.

By a combined serological and entomological survey the source of an infection can be detected in a matter of hours, whereas the alternate method requires weeks. The epidemiological surveys possible by this method can be increased manyfold, and suppressive measures can be directed more accurately and rapidly. Furthermore, such surveys can be made easily at points far from laboratory installations.

As is true of surveys conducted in the past, the percentage of positive rats was highest in food establishments. The fact that theatres are harboring infected rats and their vectors should provide some explanation for the exotic outbreaks among people who have had no known exposure to infected surroundings, and should stimulate a concerted drive to protect such mass populations from this common source of infection.

ACKNOWLEDGMENT

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THE INFECTION OF PEDICINUS ALBIDUS RUDOW THE MAGGOT'S LOUSE ON TYPHUS CARRYING MONKEYS (MACACUS SYLVANUS)

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In 1919 Arkwright and Bacot and Duncan (1) removed 150 lice (*Pedicinus longiceps*) from a typhus infected monkey (Macacus rhesus) just before death, which were inoculated subcutaneously and intramuscularly to another monkey of the same species. After an incubation period of 7 days this animal developed a febrile disease.

Dissection of the lice nourished on the infected monkeys indicated from 4–16% with rickettsiae in the digestive tract. In 1922 Atkin published the results of experiments made with Bacot in 1920 and 1921 at the Lister Institute in London, using a Polish and Irish virus (2). In this important work the authors demonstrated that the infected lice could not transmit infection by biting; a few experiments of experimental infection of *Pedicinus longiceps* on macaca monkeys are also reported.

Atkin and Bacot first took the intestinal tract of 36 Pedicinus from a *M. rhesus* on the 12th day of typhus, emulsified in normal saline, inoculating into the thigh of a macacus of the same species. The monkey developed pyrexia and was immune 8 months later to a challenge dose of infected guinea-pig brain. They observed rickettsiae in the sediment of emulsified lice which was not the case of pedicinus fed on non-infected monkeys.

Their trials of infecting guinea-pigs by the *Pedicinus longiceps*, however, were completely negative. Their first experiment consisted of the inoculation of 100 lice, from a typhus infected monkey, intraperitoneally into guinea-pigs. The animals did not react, subsequently confirmed by a challenge dose of typhus virus producing typical disease. In a second trial the authors inoculated 5 lice subcutaneously into a guinea pig obtained from a typhus monkey 15 days after the onset of fever. This time as before no infection nor immunity.

From these negative results Atkin and Bacot concluded, recognizing the risk of a considerable element of chance, that the virus of typhus lost the greater part of its virulence in passing through this unaccustomed host, the monkey louse.

In 1933 Kodama Kono and Takaposlu; Kitisato exp. med. 1933, **10**, 113 did not succeed in transmitting epidemic typhus by inoculation of ground monkey lice (Pedicinus).

This supposition is contrary to present conceptions observed by different authors and by ourselves on the behaviour of the typhus viruses in the various ectoparasites and in particular in the different species of fleas and lice for which reason we were prompted to record our experience on the infection of the monkey louse, *Pedicinus albidus Rudow*, fed on typhus infected monkeys. These results confirm the conclusions reported in previous work (3).

Experiment I. On Dec. 9 25 *Pedicinus albidus* were taken from a monkey 10 days afebrile after a typhus course. The monkey had been infected intraperitoneally with guinea pig brain of a Casablanca epidemic typhus strain. The 25 lice were ground and inoculated I.P. into guinea pig #43, which reacted, after a very short incubation period of 3 days, in usual fashion, running 6 days fever. Complement fixation using an epidemic egg antigen (Breinl strain) was strongly positive on the guinea pig serum (4+1/384). Groups of dissected lice showed numerous rickettsiae in the intestinal cells with giemsa stain.

Experiment II. 30 *Pedicinus albidus* were removed from a macaca in extremis from typhus previously infected by guinea pig brain. Complement fixation of the monkey serum at this time was (4+1/192 and 1+1/384). The lice were emulsified and inoculated intraperitoneally in guinea pig #16 (fig. 1) which after 5 days incubation reacted typically. On the 4th febrile day the animal was sacrificed and the brain passed intraperitoneally to guinea pig 41 and 42 both reacting as is apparent by figure 1. No. 42 ran the usual febrile course without orchitis, and a positive com-

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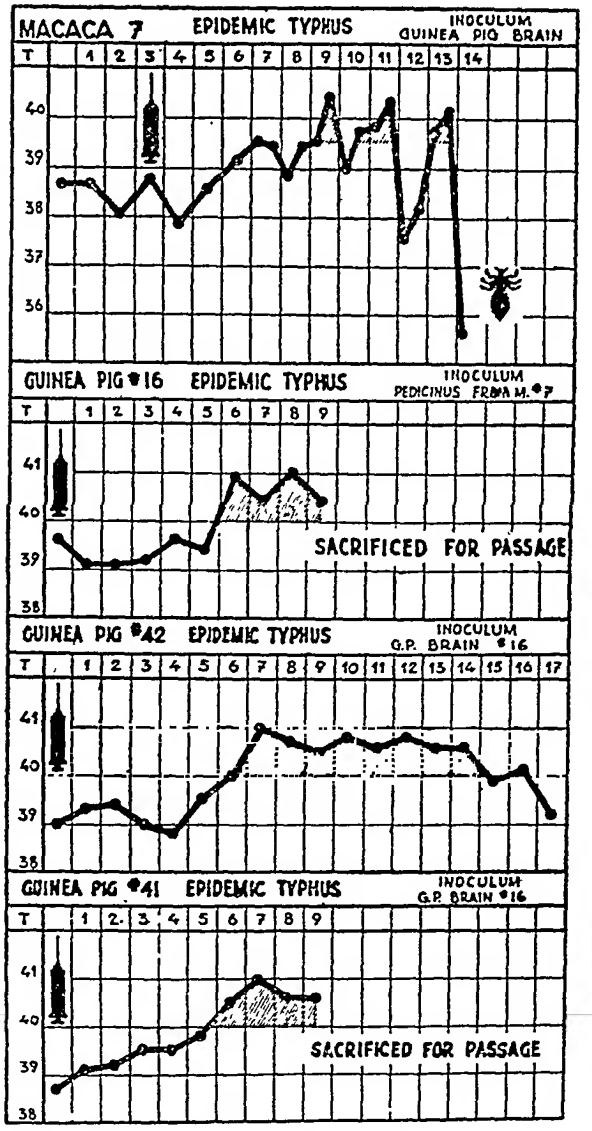

FIG. 1

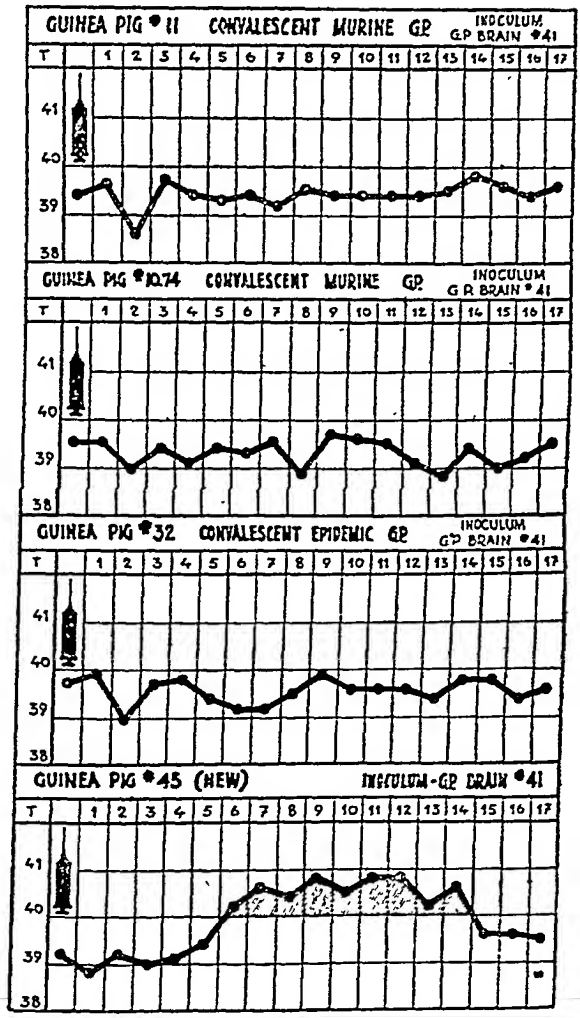

FIG. 2

plement fixation (4+1/192), No. 41 was sacrificed on the 4th febrile day, the brain used for challenging 3 convalescent murine guinea pigs, two convalescent epidemic guinea pigs and a non immune pig. The latter reacted in the usual typhus fashion, with a febrile course of 9 days and a complement fixation of (4+1/384). The murine and epidemic convalescent animals resisted infection as demonstrated in figure 2. In this second experiment groups of lice dissected showed numerous rickettsiae in the intestinal cells.

In these two experiments it is apparent that the lice (*Pedicinus albidus*) can be infected on *Macacus sylvanus* monkeys with epidemic typhus and that the virus conserves its virulence and characteristics as does the murine virus after passage through lice and the virus of epidemic typhus passed through fleas.

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AMEBIC HEPATITIS[1]

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The present discussion is meant to emphasize the importance of two aspects of amebic hepatic disease, early diagnosis and early therapy. No implication is made that these problems are newly recognized. Rogers (1) emphasized certain aspects of both as far back as 1907. We feel, however, that this presentation is justified on at least three grounds; first, the failure of a large number of the current textbooks clearly to delineate the problem or to emphasize its importance; second, records of these aspects of hepatic amebiasis are still sufficiently fragmentary to warrant further recording and correlation of case studies; and, third, inadequacies in the recognition of the picture in this country are well known.

In any disease, particularly those which are infectious in nature, demonstration of the etiologic agent places therapy upon solid ground. Amebiasis is no exception (2). Absolute diagnoses are always based upon the demonstration of the cause of the picture. However, in hepatic amebiasis, diagnosis and treatment are almost invariably carried out without demonstration of *E. histolytica* in the liver or its discharges.

Let us review the clinician's position in attaining a diagnosis in hepatic amebiasis. To establish the frequency of occurrence of findings sufficiently diagnostic to warrant consideration of hepatic amebiasis, we have reviewed the records of patients with such diagnoses at the New Orleans Charity Hospital and the Marine Hospital at New Orleans in the past five years. In all there were 81 patients, 58 with amebic hepatic abscess and 23 with amebic hepatitis. In the 58 patients with the diagnosis of abscess, 12, or approximately 20 per cent presented themselves with complaints and clinical findings which concentrated interest on the right lower pulmonary area. Both acute and chronic pictures of pleural pain, pneumonia, and, in 2 instances, pleural effusion, focused attention above the diaphragm, and 1 patient had been treated at first for atypical pneumonia and then for idiopathic pleural effusion for seven months before entering our service where hepatic changes were found. This entire group showed, aside from the pulmonary findings, evidences of hepatic involvement. In the remaining 46 cases, the presenting complaints definitely pointed to the liver, with enlargement, tenderness, at times a bulging mass, fever at times with chills, leukocytosis in 81 per cent, and, in 70 per cent of the patients, a distinct sudden onset from which the picture could easily be dated. Hence the diagnostic possibilities demanded the differentiation of bronchogenic carcinoma, bronchiectasis, basal pneumonia, atypical pneumonia, tuberculosis, empyema, pleural effusion of probable tuberculous or malignant origin with the pulmonary complaints, and, with the purely hepatic picture, of cirrhosis and malignancy of the liver, acute and chronic cholecystitis, peptic ulcer including rupture, infectious hepatitis, pyogenic liver abscess and echinococcus cyst, depending upon the nature of onset and severity and type of picture.

This list of diagnoses does not represent all the conditions with which hepatic amebiasis may be confused, but does represent the common ones assigned many of our patients before the true diagnosis was made. In our community some of these conditions are many times more common than hepatic amebiasis. This is not true in certain tropical areas and in such communities hepatic amebiasis is considered early in differential diagnosis. But, when the frequency of this condition is low in comparison to the other pictures mentioned, the possibility of its occurrence does not always come to mind. This is shown in our records. *Awareness of the possible occurrence of hepatic amebiasis* was the most important single factor in directing the early establishment of the diagnosis. Without it, diagnoses were either late, or were accidental, following the establishment of certain important data which, if the diagnosis is suspected from the clinical pictures, may be actively sought to arouse the clinical suspicion to a

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level sufficiently high to warrant therapy. These data, and their frequency as shown in our 58 patients diagnosed as abscess, were:

1. A history of diarrhea or dysentery, or its presence with the hepatic picture.... 19 (32.7%)
2. The presence of *E. histolytica* in the stools (34 patients examined)..... 10 (29.4%)
3. Roentgenologic signs of a fixed right diaphragm, bulging of the diaphragm, especially centrally and anteriorly, elevation of the right diaphragm, at times with pleural or pulmonary involvement, or evidence of hepatic enlargement.... 47 (81.0%)
4. Chocolate sauce pus either (a) from rupture, as into a bronchus, or (b) by aspiration from the pleural space or liver 27 (46.5%)..... 55 (94.8%)
5. A positive complement fixation test

Although colonic infection probably always precedes the hepatic lesion, in many instances it is not clinically active. In only 32 per cent, or approximately one-third, of the instances was a history of diarrhea obtained. In 11 per cent the onset of the picture was with diarrhea. This finding became more important clinically when it was recent and its characteristics were clear in the patient's mind. Such a finding with the hepatic picture, when the considerations in differential diagnosis given above are exhausted, warrants a therapeutic trial with emetine. Establishment of the diarrhea as a manifestation of amebiasis or the finding of positive stools without dysentery was most important, for it established *in the patient* the presence of the etiologic agent. In some reports (3) positive stools have been found in up to 70 per cent of the patients. The incidence should be higher than 29.4 per cent in our group for, although stool examinations were adequate in some patients, in others only one specimen was examined. Faust (4) has reported that in our community carrier rates are well over 10 per cent. Such high rates mean that in approximately that percentage of patients with any disease, infectious hepatitis or cirrhosis of the liver for example, the stools would be likely to be positive. As strongly suggestive as such a finding is, it is not pathognomonic of hepatic amebiasis in such patients. This finding would increase in importance in areas where carrier rates are low, but under any circumstance it establishes the presence of *E. histolytica* in the body and in the presence of the hepatic picture we have described, in the absence of any other satisfactory explanation for it, the institution of therapy for hepatic amebiasis is warranted.

In only 11, or 19 per cent, of the cases were roentgenologic findings absent. In 47, or 81 per cent, the signs were positive. In 15 (26 per cent) characteristic diaphragmatic bulging was seen; in 18 (31 per cent) elevation and impaired movement of the diaphragm, and in 14 (24 per cent) similar evidences in association with changes in the pleura and right lower lobe. As would be expected, there was a tendency for the roentgenologic findings to become more positive as the picture progressed. Such findings are important as guides in the demonstration of areas from which to aspirate pus. In 50 per cent of the cases aspiration, guided by this means and several times by bulging in the abdominal wall, demonstrated typical pus. In 11 per cent rupture occurred into a bronchus with characteristic sputum.

In 15 (26 per cent) of the 58 instances, roentgenologic findings were either not sufficiently diagnostic to warrant a tentative diagnosis to indicate an area to tap, or such evidence was altogether absent. Of the 18 with elevation of the diaphragm only, in 11 pus was obtained by tap and in 5 at operation. Collateral evidence helped settle the diagnosis in the other 2. In both diarrhea was associated, and in 1 the complement fixation test was positive.

Roentgenologic signs were, therefore, invaluable in the demonstration of hepatic involvement and spread of the disease and as an indication of the avenues for aspiration for pus. Most of the patients with characteristic roentgenologic signs are, therefore, included in the group of those with characteristic pus. It is obvious that characteristic bulging in the roentgenogram is a late finding.

Chocolate sauce pus is the closest approach to a final diagnosis, and in some instances the organism may be found in it. It is practically diagnostic of amebic hepatic abscess. In 55 of the 58 patients the diagnosis was finally settled in this way, 27 times by diagnostic tap and in the remainder through therapeutic closed or open drainage. One patient refused treatment and left the hospital; 2 others were diagnosed at postmortem examination. The fact that pus was demonstrated early in the hospitalization in 27 patients and eventually in 55 indicates the delay in diagnosis and treatment entailed by temporizing for this final diagnostic criterion.

The occurrence in combination, in the same

patient, of several of the collateral diagnostic aids discussed above, such as roentgenologic signs, diarrhea or organisms in the stools, certainly makes the presumptive diagnosis one of high degree and warrants therapeutic trial with emetine. Fifty-three per cent of the patients showed either diarrhea, organisms, or both. An outstanding deficiency in the data in our group is the lack of use of the complement fixation test. With awareness of the possible occurrence of hepatic amebiasis, the addition of this test to the above data would no doubt increase the early diagnoses and reduce the number of patients reaching the stage where pus is aspirated. Further development of and more widespread use of the complement fixation test is sorely needed in hepatic disease. In only 2 of our patients was it done and in both of these it was positive. The results, however, were not learned until therapy was well in progress, for we must send material away for this test and the problem is usually settled by the time the results are obtained. It could be a highly helpful procedure if made available.

The above data clearly indicate that the diagnosis of hepatic amebiasis approached desirable criteria only when characteristic pus with, but usually without, demonstration of the organisms, was obtained. This was always late in the disease, in our group from two weeks to seven months following the beginning of a distinctive clinical picture. It is known that at times symptoms may be absent or minimal until late disease or even rupture (5). In 50 of the patients in whom the onset of the picture could be established with some certainty, two weeks to five months, an average of six and a half weeks, elapsed from the onset until diagnosis was satisfactorily made. Based on the criterion of demonstration of characteristic pus, our diagnoses will always be late and our chances of an early preventive and therapeutic approach will be small. Amebic hepatic disease is important often long before pus is available and we must depend upon findings less diagnostic than the ideal or sit back and wait until disease has progressed to the advanced stages mentioned above. Many times, therefore, the clinician standing at the bedside of the patient should, and must, make a diagnosis in the absence of absolute data, despite the fact that he is extremely anxious for such information and may have sought it diligently. This situation is by no means unique in hepatic amebiasis. In pulmonary tuberculosis the diagnosis rests upon the demonstration of the tubercle bacillus in the sputum or in the tissues. The clinician's greatest desire, however, is to diagnose and treat the disease sufficiently early so that it is impossible to demonstrate clinically the presence of the tubercle bacillus. This desire should also apply to amebic hepatic disease, for here, as our patients demonstrate, clinical signs and symptoms are likely to develop long before it is possible to demonstrate clearly characteristic pus or the presence of *Endameba histolytica*. In any disease in which results in therapy are based primarily upon early diagnosis the absolute criteria for diagnosis are likely to be absent. Errors in diagnosis are likely to occur, but if one waits until criteria are absolutely fulfilled, great hazard is added to the life of the patient and potential benefits from treatment are greatly reduced. No better examples of these principles can be found than in the treatment of tuberculosis, carcinoma and amebic hepatic disease.

There remained 23 instances in our group in which pus was not demonstrated, nine with elevation of the diaphragm. Eleven showed evidences of colonic amebiasis and one a positive complement fixation test. One died of coronary occlusion within a month of treatment for hepatic amebiasis. Healing amebic hepatitis was found postmortem. Thus, in 14 of the 23, the criteria for diagnosis, short of demonstration of pus, were met. In 9 the diagnosis was unsupported by collateral findings. Here especially, we would like to have had complement fixation tests. All 23 reacted dramatically to emetine alone. They showed histories and, in general, the development of clinical findings in all ways similar to the group of 58 with proved abscess. As a matter of fact, a number of the patients with proven abscess gave histories and findings identical with these for some days to weeks before progression to obvious abscess formation. At such a time the physician may follow one of two courses. He may neglect to treat the patient specifically because of the inadequacy of the diagnostic findings and permit the picture to progress with the development of symptoms either until resolution takes place as a natural course of events or until the disease progresses and definite evidences, either by bulging or by rupture, indicate the presence of hepatic abscess. The second course is to treat the patient with emetine because of the high clinical suspicion of this disease and determine in a period of five or six days either the

presence of amebic hepatitis through therapeutic results or, with high probability, the absence of this disease if therapy is not effective. In the authors' opinion, both the physician and the patient should be most desirous of the latter course, for in the latter group the mortality rate was 0, while in the abscess group it was 5.2 per cent.

There is much evidence in the literature (6, 7) to indicate the importance as a clinical entity of the earlier phases of amebic hepatic disease before frank abscess can be diagnosed. Over thirty years ago Rogers described a "presuppurative" stage (1). By this he meant to differentiate the stage of abscess formation from simple amebic hepatitis. It is impossible to be certain that small single or multiple abscesses are not present in these instances without location or size which permits bulging to be recognized. Even at times when known abscess occurs (8) treatment with emetine alone may be successful. We have seen several such patients, not in this group, in whom diagnostic tap of only a few cubic centimeters of pus established the diagnosis. However, the dangers of rupture and spread, with the added mortality of these complications, demand closed drainage, preferably preceded by emetine when pus is located. Since a colonic source of amebae is assumed in all our patients, whether or not amebae were demonstrated in the stools, a course of chiniofon or diodoquin was given.

The history and physical examination uncovering the hepatic clinical picture already described do not warrant a therapeutic test until other possibilities in diagnosis are considered and evidence speaks against them, while the awareness of the possibility of amebic infection leads to establishment of the presence of colonic amebiasis, roentgenologic findings in the liver area, a positive complement fixation test, or, finally, pus, if a desirable area for tap is found. The promiscuous use of emetine, without the above attempts, is to be discouraged, for there are dangers in the use of this drug. In our group, 78 patients were treated with 6 to 12 grains of emetine hydrochloride, 1 grain daily subcutaneously or intramuscularly. Only once did untoward symptoms occur and these were cardiac. If treatment with emetine is rewarded by cure, two possibilities exist. Either the therapy and cure were coincidental, or there was a cause and effect relationship. The former is possible, especially if the diagnosis is in error, for because of inadequate criteria for diagnosis an occasional self-limited disease of the liver will be treated as amebiasis. While spontaneous cure or a remission in the disease is possible, the added risk attending the progress of the picture to a definite stage of abscess is so great that no one should permit it merely to satisfy his diagnostic curiosity. The introduction of closed drainage with emetine reduced the mortality of amebic hepatic abscess from figures over 50 per cent to 5 to 14 per cent (7). Awareness of the possibility of the occurrence of this disease when the clinical findings occurred, with early treatment with adequate doses of emetine alone, reduced this figure in our group to 0.

CONCLUSIONS

(1) The most important factor in the early recognition of amebic hepatitis is the awareness of the possible occurrence of the disease.

(2) Early diagnosis of hepatic amebiasis was shown in our group of patients to rest on collateral diagnostic findings, especially the presence of intestinal amebiasis, roentgenologic signs, and the complement fixation test. More widespread use of the latter test is indicated.

(3) Early diagnosis, before the stage in which pus could be demonstrated, in our group, led to effective treatment with emetine alone. Dangerous procedures and the stage of the disease in which prognosis is at its worst were thereby avoided, with marked reduction in the mortality rate.

(4) Diligence and care in the demonstration of evidences of amebiasis in patients with such clinical pictures, through stool and roentgenologic examinations, and the complement fixation test, together with clinical acumen in ruling out other causes of the clinical picture, will reduce both errors in diagnosis and promiscuous use of emetine to a minimum, and, despite occasional errors in diagnosis, will place the treatment of hepatic amebiasis on the desirable plane on which the treatment of early carcinoma and early pulmonary tuberculosis now rest.

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THE BLOOD PICTURE IN ASYMPTOMATIC SCHISTOSOMA MANSONI AND OTHER INTESTINAL PARASITIC INFECTIONS

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In a previous communication (1) there was reported the finding of changes in the rectal mucosa in 60.7 per cent of 155 Puerto Rican young men who were otherwise healthy except that ova of *Schistosoma mansoni* were found on fecal examination. The same group was observed to determine if any changes could be discovered in the blood picture. It has been shown by various authors (2–5) that hematological changes occur at various stages of the disease such as leukocytosis and eosinophilia in the early or invasive stage and that a typical Banti's syndrome may occur late in the course of the disease. In so far as the authors are aware no hematological studies have been reported on young adults who had no clinical symptoms and in whom the infection was discovered by routine stool examination.

At the time this work was being carried on stool examinations were made of the entire group of inductees by members of the Antilles Department laboratory and the results of that survey will be reported by Weller and Dammin (6). The method of fecal study was the Hoffman modification of the De Rivas concentration technique.

None of the persons included in this study had microfilariae in the peripheral blood.

It was found that relatively few individuals had infections with *Schistosoma mansoni* alone, and the majority of them had ova of other intestinal parasites in their stools. Thus the problem of evaluating the blood picture was complicated by multiple intestinal parasitic infections. Accordingly the individuals have been divided into groups for analysis as follows.

	persons
Schistosoma mansoni infections only	17
S. mansoni and *Tricocephalus trichiurus*	24
S. mansoni and Hookworm	16
S. mansoni, Hookworm and *T. trichiurus*	66
S. mansoni, Hookworm, *Strongyloides stercoralis*, and *T. trichiurus*	9
S. mansoni, Hookworm, *Ascaris lumbricoides*, and *T. trichiurus*	7
S. mansoni, *S. stercoralis*, Hookworm	4
S. mansoni, *T. trichiurus*, *S. stercoralis*	1
S. mansoni, *A. lubricoides*, *S. stercoralis* and *T. trichiurus*	1
S. mansoni, *S. stercoralis*	1
S. mansoni, *A. lubricoides*, Hookworm, *S. strongyloides*, and *T. trichiurus*	1
Total studied	147

It is clearly seen that while *S. mansoni* was the parasitic infection common to all of these persons there were other infections in all but 17 of the group of 147 individuals.

SCHISTOSOMA MANSONI INFECTIONS ONLY

Table 1 gives the results of the blood counts in 17 persons infected with *S. mansoni* only. The most striking feature of this table is the fact that the counts are relatively normal. There was adequate hemoglobin and the number of erythrocytes in every case was well within normal limits. There was elevation of the total leukocytes in 5 of the patients the highest count being 14,700 per cu. mm., but the differential blood counts were not markedly changed. The distribution of the eosinophiles for this group is of special interest and that data is shown graphically in Chart 1 which shows that there was complete absence of eosinophiles in 3 individuals while in 8 others there were from 1 to 5 per cent. Only 4 of those examined had between 6 and 10 per cent eosinophiles and there were 2 individuals having 11 and 14 per cent eosinophiles respectively.

SCHISTOSOMA MANSONI AND OTHER INFECTIONS

S. mansoni and Tricocephalus trichiurus. The second group of 24 individuals was that in whom the only other parasite found was *T. trichiurus.* While it is not possible to state just what clinical symptoms if any can be caused by this parasite, it is generally looked upon as an incidental laboratory finding without clinical significance. It is of interest to note that in table 2 there is marked

[1] Lt. Colonel, M.C., AUS.
[2] Captain, M.C., AUS.
[3] Captain, M.C., AUS.

similarity with the findings of *S. mansoni* infection alone. In fact this is most clearly shown in the chart where the distribution pattern of eosinophiles is almost identical for the 2 groups.

S. mansoni and Hookworm. The second group in this series was composed of 16 persons who harbored *S. mansoni* and hookworm infections. The results of the blood counts are shown in table 3 and the distribution of eosinophiles is graphically represented in the accompanying chart. As in the former groups there were no significant changes in the erythrocytes and hemoglobin. There were 4 persons in whom the total leukocytes were elevated. Eleven persons had more than 5 per cent eosinophiles.

TABLE 1

Blood counts in 17 persons infected with schistosoma mansoni but without clinical symptoms

Erythrocytes in millions	Hemoglobin in grams	Leukocytes per cu. mm.	Differential blood count—percentages				
			Polymorphonuclear	Eosinophiles	Lymphocytes	Monocytes	Basophiles
4.65	18.0	7,900	66	3	31		
4.50	15.0	8,050	56	14	30		
4.66	15.0	9,350	57	1	41	1	1
4.16	15.6	14,700	58	0	42		
4.49	15.0	8,350	53	9	38		
5.02	16.0	13,050	66	5	29		
5.41	17.9	10,800	59	2	39		
4.61	17.0	10,150	71	5	24		
5.08	16.4	9,250	57	1	42		
4.68	16.8	7,500	45	6	49		
4.43	86%	9,200	64	5	31		
4.52	90%	6,350	53	11	34	2	
4.75	94%	9,550	65	0	33	2	
5.20	16.3	8,350	47	8	42	1	2
3.91	13.3	6,850	56	3	40	1	
4.67	14.6	5,750	55	6	39		
5.16	16.8	12,200	86	0	11	2	1

S. mansoni, Hookworm and T. trichiurus. There were 66 persons with the combination of both hookworm and *T. trichiurus* infections in addition to *S. mansoni.* The blood counts of these individuals are given in detail in table 4 and the distribution of eosinophiles is shown in the chart. There were 25 of this group who had 5 per cent or less eosinophiles. A total of 19 had more than 10,000 leukocytes and 12 of these had eosinophiles.

S. mansoni, Hookworm, Strongyloides stercoralis and T. trichiurus. There were 9 persons included in this group and the details of their examinations are shown in table 5. There were 6 who had eosinophilia and 1 of these had an increase in total leukocytes.

S. mansoni, Hookworm, A. lumbricoides, T. trichiurus. This group was composed of 7 persons whose findings are shown in detail in table 6 and also in the chart for distribution of eosinophiles.

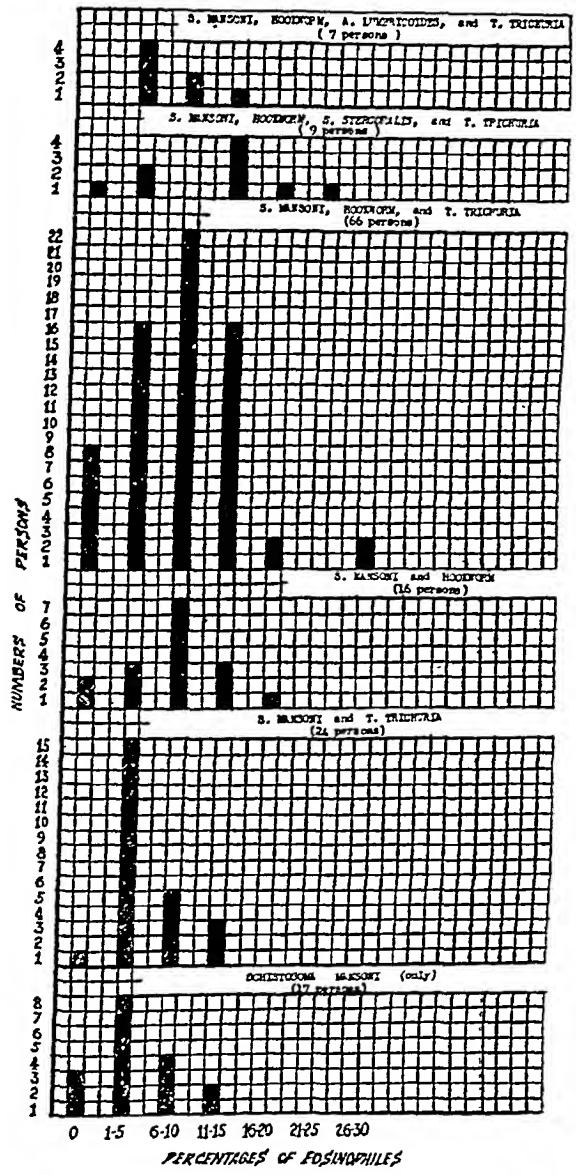

CHART 1

Distribution of Percentages of Eosinophiles Among Persons Infected With *Schistosoma mansoni* and Other Intestinal Parasites

S. mansoni and other intestinal parasitic infections not listed above. The numbers of examinations in each group were so small that these findings have been grouped into a single table namely, table 7 where the details of the examinations are given. The impressive feature of this table is that

TABLE 2

Blood counts in 24 persons infected with Schistosoma mansoni and Trichocephalus trichiurus but without any clinical symptoms

Erythrocytes in millions	Hemoglobin in grams	Leukocytes per cu. mm.	Differential blood count—percentages				
			Polymorphonuclear	Eosinophiles	Lymphocytes	Monocytes	Basophiles
4.61	15.4	4,850	62	3	35		
4.92	16.0	10,300	52	3	45		
4.25	17.2	7,350	55	3	42		
4.94	15.9	6,350	57	2	41		
4.77	15.0	6,800	57	2	41		
3.98	14.1	8,150	50	3	47		
4.49	19.0	12,000	66	2	25	7	
4.32	14.0	7,250	49	10	41		
4.24	15.0	9,750	67	0	33		
6.15	16.0	11,250	69	3	25		3
5.76	19.1	9,800	53	15	32		
4.93	15.8	7,950	46	10	44		
5.02	16.0	7,000	56	4	40		
4.71	17.1	9,950	61	12	26	1	
4.65	13.5	8,200	66	11	23		
5.29	15.5	11,650	57	9	34		
5.40	17.5	11,900	55	10	35		
4.87	17.0	12,400	49	8	43		
5.74	17.0	8,300	60	2	38		
5.53	18.0	5,050	63	4	33		
5.02	16.5	14,300	59	1	40		
4.90	15.8	11,000	80	1	19		
5.03	15.0	11,200	60	4	36		
5.05	18.5	7,550	65	3	30	2	

TABLE 3

Blood counts in 16 persons infected with Schistosoma mansoni and hookworm but without any clinical symptoms

Erythrocytes in millions	Hemoglobin in grams	Leukocytes per cu. mm.	Differential blood count—percentages				
			Polymorphonuclear	Eosinophiles	Lymphocytes	Monocytes	Basophiles
5.04	16	7,200	64	6	30		
5.29	17	11,050	62	5	33		
4.47	15	6,900	70	6	24		
5.30	18.0	7,850	62	16	22		
4.81	16.0	7,850	59	7	34		
4.77	15.0	5,500	63	0	27		
4.30	15.0	7,350	57	1	30		
4.85	14.5	5,950	54	9	43	3	
5.18	17.9	11,750	53	14	31		2
5.03	18.5	11,650	73	0	27		
5.19	15.1	12,650	62	8	30		
5.17	16.1	7,900	49	8	43		
4.50	15.5	9,900	57	14	29		
4.16	12.8	7,850	59	13	28		
4.70	15.9	7,900	55	6	37	2	
5.50	17.0	5,300	66	2	31	1	

TABLE 4

Blood counts in 66 persons infected with Schistosoma mansoni hookworm and Trichocephalus trichiurus but without any clinical symptoms

Erythrocytes in millions	Hemoglobin in grams	Leukocytes per cu. mm.	Differential blood count—percentages				
			Polymorphonuclear	Eosinophiles	Lymphocytes	Monocytes	Basophiles
5.0	16.0	7,150	65	1	34		
4.98	16.0	13,750	52	15	33		
5.00	16.0	7,800	53	2	44		
4.94	16.1	8,300	50	10	40		
4.55	17.0	10,500	58	4	38		
4.75	16.0	6,800	58	6	41		
4.21	16.0	8,250	59	6	35		
5.09	16.0	6,750	51	12	31	5	1
4.02	12.9	10,650	42	14	40	3	1
4.20	14.0	13,950	55	15	29	1	
5.65	18.5	9,900	54	16	30		
4.86	17.0	9,200	45	5	48	2	
4.16	15.0	9,200	46	8	46		
4.50	15.8	9,600	60	9	31		
4.13	14.0	7,600	43	17	40		
5.60	17.0	16,550	78	0	22		
5.10	15.1	10,350	63	7	30		
5.16	17.0	13,500	51	0	49	1	
5.25	16.8	7,100	61	0	39		
5.87	18.5	7,200	55	14	30	1	
4.95	18.0	9,200	65	0	35		
5.00	18.1	6,900	54	8	38		
4.51	14.0	8,800	73	0	27		
4.47	15.7	10,300	67	4	27		1
4.49	18.0	10,250	66	7	26	1	
4.79	17.5	14,450	71	5	23		1
4.79	14.8	9,700	37	29	34		
4.88	16.4	7,850	67	0	33		
4.82	15.8	9,300	57	3	40		
4.71	14.2	13,850	64	8	28		
5.02	16.2	13,000	54	0	46		
5.28	16.1	11,400	55	6	39		
5.07	18.0	6,200	48	8	44		
5.98	16.0	11,400	71	1	28		
5.01	15.9	11,600	56	15	29		
5.72	16.1	11,050	57	8	35		
5.25	17.0	7,000	51	5	44		
4.58	17.1	9,800	52	2	46		
5.70	18.1	8,750	36	30	34		
5.00	16.0	6,800	56	13	31		
5.10	14.5	8,700	48	13	39		
5.37	16.5	8,800	60	5	35		
5.12	16.3	5,600	56	5	39		
4.76	15.4	7,250	64	8	28		
5.83	16.3	6,150	64	6	30		
5.19	15.0	10,600	67	7	26		
5.04	17.1	6,350	55	4	41		
5.71	16.8	6,800	63	10	27		
4.90	15.8	9,200	53	7	40		
4.75	14.5	7,200	60	9	31		
5.31	16.9	7,150	69	9	22		
4.79	13.5	8,450	66	12	22		

TABLE 4—*Continued*

Erythrocytes in millions	Hemoglobin in grams	Leukocytes per cu. mm.	Differential blood count—percentages				
			Polymorphonuclear	Eosinophiles	Lymphocytes	Monocytes	Basophiles
5.03	16.5	6,900	53	8	39		
4.98	14.0	11,400	50	13	37		
5.11	15.6	8,900	59	0	41		
4.63	14.5	8,400	60	4	36		
4.98	15.5	6,750	45	6	49		
5.59	17.0	6,700	59	7	34		
4.41	15.5	7,550	49	4	47		
5.57	16.7	8,900	55	15	30		
5.03	17.0	8,800	59	11	30		
4.71	15.5	8,150	51	11	38		
5.00	16.0	9,000	67	4	29		
4.10	14.0	10,100	59	15	26		
4.80	16.0	8,300	50	15	35		
5.50	17.0	7,600	57	11	32		
5.02	17.2	6,800	56	3	41		
5.12	15.0	7,200	64	8	28		

TABLE 5

Blood counts in 9 persons infected with Schistosoma mansoni, Strongyloides stercoralis, hookworm and Tricocephalus trichiurus but without any clinical symptoms

Erythrocytes in millions	Hemoglobin in grams	Leukocytes per cu. mm.	Differential blood count—percentages				
			Polymorphonuclear	Eosinophiles	Lymphocytes	Monocytes	Basophiles
5.05	17.1	5,950	64	11	25		
5.10	17.9	7,950	55	22	23		
6.24	19.0	8,150	64	0	36		
5.86	19.9	9,250	58	14	28		
5.36	17.8	11,650	36	4	60		
4.76	16.0	7,600	64	4	32		
4.80	13.5	7,100	38	17	45		
5.70	15.1	13,700	61	13	27		
4.50	15.6	8,500	56	13	31	1	

6 of the 8 persons had eosinophilia above 5 per cent. In 1 individual there were 5 different parasitic infections, namely *S. mansoni, A. lumbricoides, Hookworm, S. stercoralis, T. trichiurus.* In this case the eosinophile count was 23 per cent.

BLOOD COUNTS IN HEALTHY PUERTO RICAN YOUNG MEN

One of us (R. R. M.) has made a study of 450 Puerto Rican young men in the Army all of whom were in the same age group as those included in the study on asymptomatic *Schistosoma mansoni* infections. In his study the stools were examined by one of the concentration methods, the urine was tested, the blood was found to be free from malaria and filaria and in all respects these individuals could be considered as healthy persons. The summary of his blood examinations are given in table 8 but the details of this exhaustive study will form a separate report.

TABLE 6

Blood counts in 7 persons infected with Schistosoma mansoni, Ascaris lumbricoides, Tricocephalus trichiurus and hookworm but without any clinical symptoms

Erythrocytes in millions	Hemoglobin in grams	Leukocytes per cu. mm.	Differential blood count—percentages				
			Polymorphonuclear	Eosinophiles	Lymphocytes	Monocytes	Basophiles
4.45	17.0	7,500	62	7	31		
4.67	15.8	6,050	55	5	40		
5.60	16.9	10,450	54	11	34	1	
4.80	15.5	10,400	73	5	22		
5.17	17.9	11,700	67	9	29		
5.11	16.1	7,750	62	2	36		
5.60	17.0	8,800	67	2	31		

SUMMARY AND CONCLUSIONS

(1) A study of the blood picture was made of 147 Puerto Rican young men who had no clinical symptoms but in whom the infection with *Schistosoma mansoni* as well as other intestinal parasites such as *Hookworm, Trichocephalus trichiurus, Strongyloides stercoralis* and *Ascaris lumbricoides* were found on routine fecal examination.

(2) There were 17 individuals infected with *S. mansoni* alone. In this group 5 had leukocytosis and 6 had eosinophilia. The highest count was 14 per cent and there were 3 persons who had no eosinophiles.

(3) The only alterations from normal in the remainder of the group were increases of varying degree of the percentages of eosinophiles and slight leukocytosis. The highest eosinophilia was 30 per cent. The highest counts being in those in whom there were *S. mansoni, Hookworm,* and *T. trichiurus.* There were 17 individuals of the 147 studied in whom no eosinophiles were found.

(4) In spite of the various parasitic infections present in the group of 147 young men, the blood picture shows a striking resemblance to the blood findings in 450 healthy Puerto Rican males who were free from intestinal and blood parasites.

TABLE 7

Blood counts in persons infected with Schistosoma mansoni and other parasites as indicated but without any clinical symptoms

PARASITES	ERYTHROCYTES IN MILLIONS	HEMOGLOBIN IN GRAMS	LEUKOCYTES PER CU. MM.	DIFFERENTIAL BLOOD COUNTS—PERCENTAGES				
				Polymorphonuclears	Eosinophiles	Lymphocytes	Monocytes	Basophiles
S. mansoni	4.11	13.0	7,850	57	0	43		
S. stercoralis	5.14	16.5	10,250	66	0	34		
Hookworm	4.93	17.0	10,450	51	6	43		
	5.90	16.5	7,750	48	8	44		
S. mansoni *T. trichiurus* *S. stercoralis*	4.61	16.5	11,800	51	13	30	5	1
S. mansoni *A. lumbricoides* *T. trichiurus* *S. stercoralis*	4.80	17.1	8,300	54	7	38	1	
S. mansoni *S. stercoralis*	4.07	13	6,050	57	8	35		
S. mansoni *A. lumbricoides* Hookworm *T. trichiurus* *S. stercoralis*	5.03	15.0	7,650	46	23	28	3	

TABLE 8

Blood counts in 450 healthy Puerto Rican males

(Capt. R. Rodriguez-Molina, M.C.)

	MINIMUM VALUES	MAXIMUM	AVERAGE
Erythrocytes	4,000,000	5,900,000	4,810,000
Hemoglobin grams	12.6	19.4	15.4
Total Leukocytes	3,800	21,000	8,900
Polymorphonuclear Neutrophils	27%	62%	51%
Lymphocytes	19	51	26
Eosinophiles	1	28	7
Monocytes	1	12	3

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CHINESE SOY BEAN SAUCE AS A TRANSMITTING AGENT OF BACTERIAL GASTRO-INTESTINAL INFECTIONS

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It is believed that some popular accessary food articles and flavors in the Chinese diet may have a part in the transmission of intestinal parasitic and bacterial infections. Chang and Chin found (Chinese Med. Jour., **61**: 63, 1943) that the Chinese pickled vegetables could transmit Ascaris lumbricoides. The purpose of the present study is to determine whether the Chinese soy bean sauce plays any role in the transmission of diseases caused by organisms of the typhoid-dysentery group. Attempts are made (1) to see whether natural contamination of the sauce with these organisms occurs and (2) to ascertain how long these organisms when inoculated into this medium can survive in it. Our preliminary findings which we deem of sufficient interest are hereby reported.

The sauce is a water extract of soy beans in concentrated salt solution. In its preparation, the soy beans are first boiled in water and then exposed to air for fermentation. The well fermented beans are then soaked in salt solution, consisting of 10–20 lbs. of common salt in about 100 lbs. of water. The latter is generally taken from nearby shallow wells and is not heated nor sterilized in any way. This mixture is put aside for a few days, and then filtered through a bamboo sieve. The filtrate thus obtained is the soy bean sauce. It is either sold immediately or stored in big earthenware receptacles for a period varying from a few days to many months. These receptacles are frequently exposed to air and sunlight. In the households, the sauce is either used for cooking or consumed raw. In the latter case, the food articles, especially the cold varieties, are soaked in the sauce to get additional flavours.

METHOD OF STUDY

1. Collection of samples

Samples were secured from various soy bean sauce shops distributed throughout the city. From each shop three samples were obtained. The most popular shops were selected. Each sample, consisting of about 20 cc. of sauce, was collected in a sealed sterile tube, care being taken to prevent contamination.

2. Reaction and salt concentration of the samples

Because it was impossible to determine the pH values of the sauce by the colorimetric method, owing to its dark color, the reaction was tested by litmus paper. The salt concentration of the sauce was determined by titration with 0.1 normal silver nitrate solution. One cc. of the sauce was diluted to about 400–500 cc. with double distilled water and then titrated against the above mentioned solution. The end point was taken, when no more fresh white precipitate was formed. The salt concentration could be easily calculated on the assumption that all the chloride was combined with sodium. For instance, 1 cc. of 0.1 normal silver nitrate corresponds to 0.0058 gram of sodium chloride. The latter factor (0.0058) times the volume in cc. of 0.1 normal silver nitrate used would give the actual weight of sodium chloride in any particular sample.

3. Isolation of pathogenic organisms from the sauce

One drop (equal to 0.05 cc.) of each sample of the sauce was inoculated into a China blue rosalic acid agar plate, and 1 cc. into 9 cc. of meat infusion broth (pH 7.6). The media were incubated at 37°C. and examined for growth 24 and 48 hours later. If positive, the organisms were identified accordingly.

4. Survival of inoculated organisms in the sauce

One loopful of *E. typhosa*, *S. dysenteriae* (Shiga) or *V. cholerae* was inoculated into one of the three tubes, each containing 1 cc. of the sauce. Two sets were prepared. One set of the inoculated tubes was kept in the incubator (36–38°C.), and the other set at room temperature. After 24 hours, cultures were made on China blue rosalic acid agar plate; if no growth in the plate was obtained, the inoculated sauce in each separate tube was transferred to meat infusion broth to see

whether the inoculated organisms could be recovered. If positive, transfers were made to new media at various intervals to detect the surviving periods of the respective organisms. The organisms recovered were checked accordingly by sugar reactions and agglutination against specific diagnostic sera.

TABLE 1

Pathogenic organisms isolated from soy bean sauce

SAMPLE NO.	DATE (1915)	REACTION TO LITMUS PAPER	SALT CONCENTRATION IN %	ORGANISMS ISOLATED: Grown in China blue agar plate	Grown in meat infusion broth	Morphology	Gram stain	Motility	Sugar reactions: Lactose	Glucose	Manite	Maltose	Methyl red test	Agglutination against diagnostic serum	Conclusion on species of organism.
A_1	Sept. 8	Acid	8.1	0	+	Bacilli	Neg.	−	A (after 24 hrs.)	A	A	A		*S. paradysenteriae sonnei* dilution 1/1,280	*S. paradysenteriae sonnei*
A_2	8	Acid	11.7	+	+	Bacilli	Neg.	+	0	A	A	A		*E. typhosa*; dilution 1/320	*E. typhosa*
A_3	8	Acid	14.0	0	0										
B_1	Sept. 22	Acid	10.4	0	+	Bacilli	Neg.	+	0	AG	AG	AG			Salmonella bacilli*
B_2	22	Acid	16.2	0	+	Bacilli	Neg.	+	AG	AG	AG	AG	+		Coliform organism
B_3	22	Acid	11.6	0	+	Bacilli	Neg.	+	AG	AG	AG	AG	+		Coliform organism
C_1	Sept. 28	Acid	12.1	0	0										
C_2	28	Acid	13.5	0	+	Bacilli	Neg.	+	AG	AG	AG	AG	+		Coliform organism
C_3	28	Acid	12.0	0	+	Bacilli	Neg.	+	AG	AG	AG	AG	+		Coliform organism
D_1	Oct. 1	Acid	14.3	0	0										
D_2	1	Acid	11.6	0	0										
D_3	1	Acid	11.0	0	0										
E_1	Oct. 5	Acid	11.8	+	+	Bacilli	Neg.	+	0	A	A	A		*E. typhosa* dilution 1/1,280	*E. typhosa*
E_2	5	Acid	16.2	0	0										
E_3	5	Acid	11.3	0	+	Bacilli	Neg.	−	0	A	A	0		*S. paradysenteriae flexner* 1/1,280	*S. paradysenteriae flexner*
F_1	Oct. 12	Acid	17.9	0	0										
F_2	12	Acid	15.6	0	+	Bacilli	Neg.	+	AG	AG	AG	AG	+		Coliform organism
F_3	12	Acid	12.4	0	0										
G_1	Oct. 15	Acid	13.9	0	0										
G_2	15	Acid	19.4	0	0										
G_3	15	Acid	15.6	0	0										
H_1	Oct. 19	Acid	17.0	0	0										
H_2	19	Acid	16.0	0	0										
H_3	19	Acid	13.3	0	0										
I_1	Oct. 25	Acid	11.0	0	0										
I_2	25	Acid	14.1	0	+	Bacilli	Neg.	+	AG	AG	AG	AG	+		Coliform organism
I_3	25	Acid	10.9	0	0										
J_1	25	Acid	17.8	0	0										
J_2	25	Acid	16.0	0	0										
J_3	25	Acid	13.3	0	0										

A = Acid.
G = gas.
* Species not identified, for specific sera were not available.

RESULTS

The samples analysed were distributed practically throughout the city. Altogether, thirty samples of soy bean sauce were examined (table 1). Six samples (B_2, B_3, C_2, C_3, F_2, I_2), were found to be contaminated with *E. coli*, presumably of fecal origin, two samples (A_2, E_1), with *E. typhosa*, two samples with *S. paradysenteriae*, (E_3 with flexner and Ai with sonnei type), and one sample (B_1), with an unidentified bacillus of the Salmonella group. Evidently, the present examination must have missed a number of positive findings in samples with only light contamination; yet typhoid-dysentery organisms were fairly commonly found. Furthermore, *E. typhosa* were present even in 0.05 cc. of the sample inoculated directly on agar plate, which

would certainly indicate a rather heavy contamination. As the soy bean sauce is very frequently consumed raw, its contamination with these organisms may seriously endanger health. Although the salt concentration of the samples ranged from 8.1% to 19.4% and the reactions were always acid, pathogenic organisms seemed to survive well in these markedly hypertonic and acid solutions.

temperature varying from 36° to 38°C. *E. typhosa* lived longer than *S. dysenteriae* and the longest period of life for the former organisms in the sauce so far noted was 29 days as in the case of sample E_3. Four to twelve days of survival was fairly common. For example, Samples D_1, and D_3 lived for 4 days; A_2, 10 days; and E_1, 12 days. *V. cholerae* lived neither at room temperature nor in incubator in the inoculated specimens for more than 24 hours.

TABLE 2

Time of survival of organisms inoculated into the sauce

SAMPLE NO.	DATE (1943)	SALT CONCENTRATION IN %	REACTION TO LITMUS	SURVIVAL OF INOCULATED ORGANISMS AFTER 24 HOURS					
				Room temp. (25–17°C.)			Incubator temp. (36–38°C.)		
				E. typhosa	*S. dysenteriae* (shiga)	*V. cholerae*	*E. typhosa*	*S. dysenteriae* (shiga)	*V. cholerae*
A_1	Sept. 8	8.1	Acid	+	+	–	–	–	–
A_2	8	11.7	Acid	+	+	–	–	–	–
A_3	8	14.0	Acid	+	–	–	–	–	–
B_1	Sept. 22	10.4	Acid	+	–	–	–	–	–
B_2	22	16.2	Acid	+	–	–	–	–	–
B_3	22	11.6	Acid	+	–	–	–	–	–
C_1	Sept. 28	12.1	Acid	–	–	–	–	–	–
C_2	28	13.5	Acid	–	+	–	–	–	–
C_3	28	12.0	Acid	–	+	–	–	–	–
D_1	Oct. 1	14.3	Acid	+	+	–	–	–	–
D_2	1	11.6	Acid	+	+	–	–	–	–
D_3	1	11.0	Acid	+	+	–	–	–	–
E_1	Sept. 5	11.8	Acid	+	+	–	–	–	–
E_2	5	16.2	Acid	+	+	–	–	–	–
E_3	5	11.3	Acid	+	+	–	–	–	–
F_1	Sept. 12	17.9	Acid	+	+	–	–	–	–
F_2	12	15.6	Acid	+	+	–	–	–	–
F_3	12	12.4	Acid	+	+	–	–	–	–
G_1	Sept. 15	13.9	Acid	+	+	–	–	–	–
G_2	15	19.4	Acid	+	+	–	–	–	–
G_3	15	15.6	Acid	+	+	–	–	–	–
H_1	Sept. 19	17.0	Acid	–	–	–	–	–	–
H_2	19	16.0	Acid	+	–	–	–	–	–
H_3	19	13.3	Acid	+	–	–	–	–	–
I_1	Sept. 25	11.0	Acid	–	–	–	–	–	–
I_2	25	14.1	Acid	+	+	–	–	–	–
I_3	25	10.9	Acid	+	+	–	–	–	–
J_1	25	17.8	Acid	+	+	–	–	–	–
J_2	25	16.0	Acid	–	+	–	–	–	–
J_3	25	13.3	Acid	–	–	–	–	–	–

The results of inoculation of specific organisms into the various samples of the sauces are tabulated in table 2. It is interesting to note that after having been inoculated into the soy bean sauce, both *E. typhosa* and *S. dysenteriae* could survive for at least 24 hours in most instances, and their survival was much better at room temperature, ranging from 17° to 25°C., than at the incubation

DISCUSSION

The fact that the Chinese soy bean sauce is very commonly contaminated by organisms of the typhoid and dysentery group is certain. A number of interesting questions, however, need further

investigation, for example: When and where does the contamination take place? Does it occur during the process of manufacturing, storage or distribution? Why do the typhoid and dysentery bacilli live better at the room temperature than in the incubator? Would the hypertonicity or other constituents in the sauce in any way modify the virulence of the contaminated organisms? Some of these points shall be studied in further experiments.

SUMMARY

1. This experiment was made to determine whether the Chinese soy bean sauce plays any part in the dissemination of diseases caused by organisms of the typhoid and dysentery group.

2. Thirty samples of sauces were examined; 35% of these samples showed evidence of contamination either with *E. coli*, *E. typhosa*, *S. dysenteriae*, or *Salmonella* bacilli.

3. The hypertonicity and acidity of the samples have no significant germicidal effect on these organisms, and *E. typhosa* inoculated into the sauce can survive for many days.

4. The fact that the soy bean sauce is frequently consumed raw and often contaminated with the typhoid-dysentery group of organisms suggests the possibility that they play a part in the dissemination of intestinal infections in this country.

TRICHINELLA SKIN TESTS IN TUBERCULOSIS SANATORIUMS, HOSPITALS FOR MENTAL DISEASES, AND GENERAL HOSPITALS

A Comparison of the Results in Tuberculous and Non-tuberculous Patients

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In a recent study conducted in general hospitals and other institutions, it was discovered that among the patients with tuberculosis confined in sanatoriums there was a significantly higher incidence of positive trichinella skin tests than among the patients in general hospitals (1). The present study was undertaken to determine if the high incidence of positive skin tests in persons with tuberculosis (14.3 per cent as opposed to 7.1 per cent in persons without tuberculosis) could be accounted for by unrecognized subclinical institutional epidemics, or if some biologic factor is involved.

In order to keep the institutional factor constant, the control nontuberculous group was chosen from patients confined in hospitals for years. This group was, of necessity, composed chiefly of psychiatric patients, and it was found that these patients themselves gave abnormal responses to the skin tests. Because of this unexpected finding, the experiment could not answer clearly either of the questions posed above. The suggestive evidence that an unrelated disease process may alter the interpretation of the trichinella test warrants the presentation of the inconclusive data and the discussion of their possible implications, with the hope of stimulating further investigation.

MATERIAL

A total of 1586 patients were subjected to skin test. The distribution of the patients by institutions is seen in table 1, which is divided into tuberculous and non-tuberculous groups. The diagnosis of tuberculosis, as made at the sanatoriums, was accepted without question, and no attempt was made to detect previously unrecognized tuberculosis in the control group. Since the incidence of positive trichinella skin tests varies with age (2), the initial groups were matched as closely as possible in age and sex, but this proved to be an unnecessary refinement. All of the patients had been confined to institutions for a period of months or years. Some of the institutions admit exclusively white or negro patients; others admit both.

The tuberculous and non-tuberculous groups were analyzed according to sex, age, race, residence in rural and urban areas, the duration of confinement in an institution, and the extent of reaction to the test. The data are summarized in tables 2, 3, 4, and 5.

TABLE 1
Incidence by institutions

INSTITUTION	TESTED	POSITIVE	%
North Carolina Sanatorium	402	84	20.9
Western North Carolina Sanatorium	155	19	12.3
Guilford County Sanatorium	117	20	17.1
Tuberculous patients	674	123	18.3
Raleigh State Hospital	331	44	13.3
Morganton State Hospital	330	71	21.5
Forsyth County Home for Infirm	87	5	5.8
Goldsboro State Hospital	164	37	22.6
Non-Tuberculous patients	912	157	17.2
Total	1586	280	17.7

When it became apparent that the primary disease from which the patient suffered might be a greater factor in the production of positive reactions than was the length of confinement in an institution, the figures previously reported in the study of patients in general hospitals for *acute* illness, where confinement is too short to be a factor, were added (1). The data have been rearranged in table 1-A according to the type of disease treated at each institution.

METHOD

In all instances a 1:10,000 dilution of trichinella extract and a phenolized phosphate buffered saline control solution were used.[1] An area on the flexor surfaces of both forearms was cleaned with alcohol, and two workers simultaneously injected 0.02 to 0.03 cc. of the test solution intradermally into one arm and the same amount of control solution into the other arm. The reactions were read fifteen to twenty minutes and twenty-four hours later; the degree of erythema and wheal, and the presence or absence of pseudopods were noted. The reaction was recorded as positive if the diameter of the area of erythema with induration, or wheal with pseudopods, exceeded that of the injected bleb by 5 mm., either at twenty minutes or twenty-four hours. The positive reactions were graded from 1 to 4 plus. If the reaction exceeded the diameter of the injected bleb by less than 5 mm., it was recorded as doubtful (±) and was counted as negative in the statistical analysis. If the control injection also produced a reaction, the test was counted as negative in the statistical analysis.

TABLE 1-A*

Incidence by type of disease in Institution

INSTITUTION	TESTED	POSITIVE	%
North Carolina Sanatorium	402	84	20.9
Western North Carolina Sanatorium	155	19	12.3
Guilford County Sanatorium	117	20	17.1
Tuberculosis Sanatoriums	674	123	18.3
Raleigh State Hospital	331	44	13.3
Morganton State Hospital	330	71	21.5
Goldsboro State Hospital	164	37	22.6
Psychiatric Hospitals	825	152	18.4
Forsyth County Home for Infirm	87	5	5.8
Chronic general hospital	87	5	5.8
Baptist Hospital	278	16	5.8
Forsyth County Hospital	57	8	14.0
Acute general hospitals	335	24	7.2
Total general hospitals	422	29	6.9

* Included are data on general hospitals which has been previously published (1). These data vary in minor detail from the previous report, since slightly more stringent criteria were used in the recalculation.

[1] The trichinella antigen and control solutions used in this study were supplied by Lederle Laboratories, Pearl River, New York.

New syringes and needles were obtained for the skin tests and these were not used for any other purpose. One syringe and needle were always used for the test solution and another for the control; the syringes and needles were never interchanged. After being used, they were washed with distilled water only, placed in marked tubes and sterilized in an autoclave.

TABLE 2

Race, sex, residence

	TUBERCULOUS			NON-TUBERCULOUS			TOTAL		
	Tested	Positive	%	Tested	Positive	%	Tested	Positive	%
White	485	88	18.1	706	115	16.3	1191	203	17.0
Negro	189	35	18.4	206	42	20.4	395	77	19.5
Male	282	47	16.7	358	65	18.2	640	112	17.5
Female	392	76	19.4	554	92	16.6	946	168	17.8
Rural	372	73	19.4	563	94	16.7	935	167	17.9
Urban	302	50	16.5	349	63	18.1	651	113	17.4

No attempt was made to correlate the trichinella skin test with the tuberculin skin reaction, since the latter could not be done on the tuberculous patients.

RESULTS

General

Of the 1586 patients in the series 280 (17.7 per cent) gave positive tests, as compared with 10 per cent of 700 patients in the previously reported series (1). The incidence was essentially the same in white patients and Negroes, in males and females, and in rural and urban residents (table 2). The patients who had spent most of their lives in communities with a population of 2,500 or less were classified as rural inhabitants.

The incidence of positive reactors was found to decrease with advancing age (table 3). (The

tuberculous groups 60 years of age or older must be discounted because of their small size.) The incidence of positive tests in relation to the duration of confinement in an institution was also determined (table 4). Discounting the very few tuberculous patients who had been confined for five years or more, the incidence of positive reactors was found to reach a peak in patients who had been in an institution between eighteen and twenty-nine months.

TABLE 3

Incidence by age

AGE	TUBERCULOUS			NON-TUBERCULOUS		
	Tested	Positive	%	Tested	Positive	%
10–19	63	18	28.6	83	27	32.5
20–29	278	54	19.4	347	69	19.9
30–39	181	32	17.7	223	36	16.2
40–49	82	10	12.2	120	13	10.8
50–59	47	5	10.6	64	9	14.1
60–69	19	3	15.8	34	0	0
70–79	4	1	25.0	37	3	8.1
Unknown	0	0	0	4	0	0
Total.....	674	123	18.3	912	157	17.2

The results were further analyzed by the degree of the reaction (table 5). The results are more clear-cut, with fewer false positive and unusual reactions, in the tuberculous group. Since 21.3 per cent of the non-tuberculous patients who were examined for the immediate reaction were not rechecked for the late reaction, the figures for the twenty-four hour reading are of less significance in this group than in the tuberculous patients.

Of the 280 positive tests, 250 were immediate reactions. In 20 of these the wheal with erythema remained for twenty-four hours as an area of *persistent* erythema. In 30 patients, the reaction was *delayed* in type and did not appear until the following day. In 11 psychiatric patients in two mental institutions, positive reactions to the control solution were observed at twenty-four hours, although the test solution gave negative results; this finding had not previously been noted.

Only one of the patients with a positive reaction

TABLE 4

Incidence by duration of stay in an institution

DURATION OF STAY	TUBERCULOUS			NON-TUBERCULOUS		
	Tested	Positive	%	Tested	Positive	%
1–5 mos.	198	21	10.6	140	30	21.4
6–11	197	44	22.3	105	18	17.2
12–17	98	20	20.4	92	12	13.4
18–23	51	13	25.5	47	13	27.7
24–29	46	15	32.6	50	12	24.0
30–36	23	5	21.7	43	7	16.3
3–5 yrs.	31	1	3.2	158	29	18.3
5–10	5	2	40.0	134	22	16.4
10–···	7	2	28.6	95	8	8.5
Unknown	18	0	0	48	6	12.5
Total.....	674	123	18.3	912	157	17.2

TABLE 5

Extent of reaction

	TUBERCULOUS				NON-TUBERCULOUS			
	20 min.	%	24 hrs.	%	20 min.	%	24 hrs.	%
0	531	78.7	600	89.0	687	75.4	607	66.7
±	9	1.4	12	1.8	48	5.3	59	6.5
1+	65	9.7	18	2.6	84	9.2	25	2.7
2+	33	4.9	4	0.6	29	3.2	4	0.4
3+	11	1.6	0	0	21	2.3	0	0
4+	2	0.3	0	0	4	0.4	0	0
±T ±C	0	0	1	0.2	8	0.8	4	0.4
+T +C	23	3.4	0	0	26	2.9	8	0.8
0T +C	0	0	0	0	3	0.3	11	1.2
Not read	0	0	39	5.8	2	0.2	194	21.3
Total.....	674	100.0	674	100.0	912	100.0	912	100.0

T signifies Test; C signifies Control.

gave a history which suggested clinical trichinosis.

No untoward reaction to the test was noted in any patient.

Patients with known allergic histories reacted in the same manner as non-allergic individuals.

Institutions

The incidence of positive reactions at each institution is shown in table 1-A. The variation is great—5.8 to 22.6 per cent—with the highest and lowest figures in the non-tuberculous hospitals. The low incidence among the small group of patients who were confined semi-permanently in a single general hospital for chronic invalids seems to indicate that the *disease process* may be more important than the length of confinement.

Of the patients in tuberculosis sanatoriums 18.3 per cent gave positive tests, as compared with an incidence of 17.7 per cent among patients in other institutions. If the data in table 1-A are used, the incidence in tuberculosis sanatoriums and psychiatric hospitals is found to be essentially the same. If the factor of institutionalization is ignored, the incidence in the tuberculous group (18.3 per cent) may be compared with that in the acute and chronic *general* hospital group, (6.9 per cent).

DISCUSSION

General conclusions

Sex, race, and residence in rural or urban areas (table 2) appeared not to be significant factors. The group in each institution was analyzed from every standpoint possible, but since no factor of apparent significance was discovered, only the figures shown in the tables are given.

Although in autopsy studies the incidence of trichinella infections rises with increasing age, the incidence of positive skin tests in this series, as in another series (2), decreased with increasing age. This finding may indicate either that skin sensitivity is lost seven to ten years after the infection, or that skin reactivity is less in the aged.

The eosinophil count, which was available in the tuberculosis sanatoriums and in Forsyth County Hospital and Home for the Aged and Infirm, could not be correlated with the skin reaction, and hence is probably of no help in the diagnosis of subclinical trichinosis. This conclusion is in agreement with that of Gould (2).

Skin test vs. autopsy findings

The most accurate method of diagnosis is still the demonstration of the larva of the parasite in the tissue of the host. This is easily done at autopsy, when muscle from several portions of the body can be obtained and studied by the muscle press technique—which is especially useful in detecting old infections, including dead larvae or calcified cysts—, or by the digestion technique, which detects only the living larvae. These methods are definitely limited in their application to living patients; the amount of muscle removed by biopsy is small, and is best studied in the muscle press. Most surveys of the incidence of trichinosis in various parts of the country have been done on autopsy material.

Tissue methods may miss light or early infestations. The skin test should detect infection in any muscle and is less dependent on the degree of infestation. It usually becomes positive early in the disease, but may remain negative in overwhelming infections. The skin test has been used more widely for the diagnosis of trichinosis in suspected clinical cases than for the determination of the incidence of the disease in random surveys. Gould has reviewed the uses and limitations of the skin test, and has shown that in a large group of individuals studied in Detroit, the incidence of positive skin tests (5.9 per cent) was considerably lower than the incidence of trichinella infestations found at autopsy (22.6 per cent) (2). The close correlation in a similar study of a smaller group in Washington has been reported by Schapiro et al. (3). The incidence of positive skin tests in this geographic area (17.7 per cent) is considerably higher than the incidence of trichinella infestations found previously in a series of autopsies (2.8 per cent) (4). Thus, each of these three studies leads to a different conclusion regarding the reliability of the skin test. Light subclinical infections may be a significant factor in causing this discrepancy, but it is also possible that some intercurrent disease process in the patient may alter the reliability of the skin test.

Types of reactions to skin tests

Spink (5) first observed the two types of reactions to injected trichinella antigen: (1) an immediate response with [reticulated] erythema and wheal (fig. 1), and (2) a delayed response with [solid] erythema and induration (fig. 2). From

observations of patients known to have been recently infected, he concluded that the delayed response signified an infection of seventeen days or less, and that the reaction changed to the immediate type after this time. We have not had an opportunity to observe known recent infections. Some additional explanation must be sought to account for the delayed reaction, however; for no sharp rise such as would be expected to follow a recent epidemic was noted in any institution in the group with the shortest duration of stay. The persistence of a positive test for twenty-four hours has rarely been commented on before. We do not know the significance of this finding, although it may be an intermediate stage in the transmission from the delayed to the immediate response.

In an attempt to discover an explanation for the delayed and persistent reactions, 13 patients at the North Carolina (Tuberculosis) Sanatorium, were retested two and one-half months after the initial test. Of 7 patients who at first gave a *delayed* reaction, 2 now gave immediate positive reactions, 2 again gave delayed positive reactions, and 3 gave no reactions at twenty minutes or twenty-four hours In 6 patients whose initial positive reaction *persisted* for twenty-four hours, 2 tests were positive at thirty minutes and negative at twenty-four hours, 2 were negative at thirty minutes and positive at twenty-four hours, and 2 were negative at each reading. These patients had been in this sanatorium for periods ranging from five months to four years and ten months All were on a diet prepared in a central

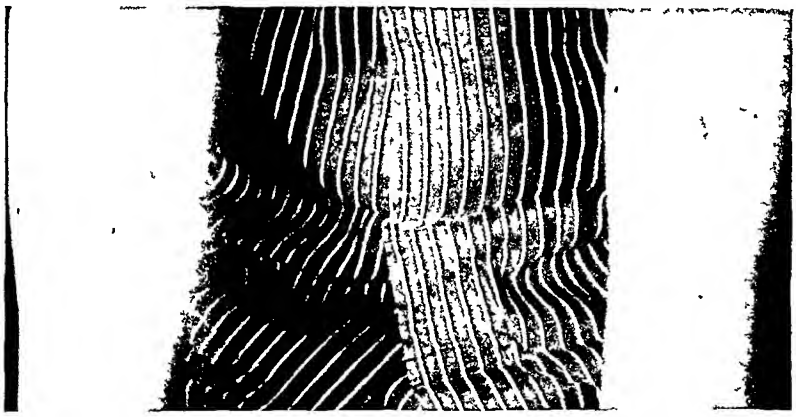

FIG. 1. Immediate reaction at twenty minutes to 0 02 cc. 1:10,000 trichinella extract injected intradermally. Pseudopods and reticulated erythema are seen.

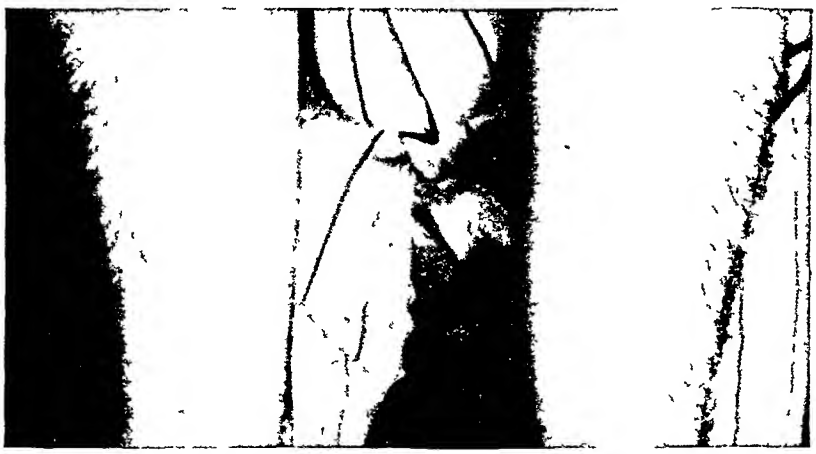

FIG. 2. Delayed reaction at twenty-four hours to 0 02 cc 1:10,000 trichinella extract injected intradermally. The erythema was solid and accompanied by slight induration.

kitchen, and no known epidemic had occurred; so that some factor other than an early infection seems to be involved. These groups are small in relation to the total number of patients, and hence any conclusion drawn from these tests would be of dubious value. We were unable to relate the delayed and persistent reactions to confinement in any particular institution or to the presence or absence of tuberculous infection.

Many individuals who now have a strongly positive skin test, before entering an institution ate large quantities of barbecue which was always thought to have been well cooked. Whether skin sensitivity may be acquired by the ingestion of dead trichinae has not been determined by biologic tests.

The possibility that tuberculosis and psychiatric disturbances may interfere with the accuracy of the skin test for trichinella has not been discussed before.

Incidence in North Carolina vs. United States

The low incidence of trichinella found in the previous study of 105 diaphragms obtained at *autopsy* in North Carolina (2.8 per cent) contrasts with an incidence of 27.6 per cent in Boston and a national average of 13–17 per cent. This finding was attributed to the food habits of people in this geographic area (4). Except around cities, hogs are fed garbage from local farms only, so that epidemics are small and are usually confined to single families. Pork is usually cooked thoroughly in the South, and little smoked sausage or other uncooked prepared pork products are used. The infections detected at autopsy were light; many more very light old infestations of less than 1 larva per gram of muscle may have been missed, although both muscle press and digestion techniques were used. The only section from which a similar low incidence has been reported is New Orleans.

The incidence of positive skin tests in our series (17.7 per cent) compares closely with the incidence of trichinella as determined at autopsy for the country as a whole (13–17 per cent), but such a comparison may be invalid for the reasons discussed below. The incidence of positive skin tests in North Carolina (17.7 per cent) is definitely higher than that reported from Detroit (5.9 per cent) (2), but compares closely with an incidence of 18.3 per cent in a smaller series in Washington, D. C. (3). It has not been possible from the published data to evaluate the factor of the primary disease process in these two series.

Institutional factor

Whether the apparent increase of positive trichinella skin tests in tuberculous patients is of true biologic significance or is connected with subclinical institutional epidemics still has not been definitely determined because of the unexpected findings in the psychiatric control group. In the previous study, the non-tuberculous patients were in an institution too short a time for this factor to be of significance. That the duration of stay in an institution may be significant is indicated by the fact that the incidence of positive reactions begins to rise in the group of tuberculous patients who had been in a sanatorium for six to eleven months and reaches a peak in the group who had been confined for twenty-four to twenty-nine months; in the non-tuberculous patients, the peak is reached at the eighteen to twenty-three month period, with a secondary rise at the five to ten year period, as shown in table 4.

In each of the institutions food is purchased in large lots. In some institutions all food is cooked in a central kitchen and is distributed by hot tables or steam carts to individual buildings on the grounds. In others, food is cooked in the various buildings, although it is supplied from a single central source. In most institutions some of the pork used is obtained from hogs raised on the grounds. In only one instance is garbage brought in from a neighboring town and fed to the hogs; in all instances garbage from the institution is used and is fed without cooking. In all institutions the portions of meat cooked are larger than in private homes, and hence the center may be inadequately cooked and infectious, even though the surface is thoroughly done.

The low incidence of positive skin tests (7.2 per cent) in the *acute* general hospital group of patients in table 1-A, who were in an institution for days rather than months or years, would also seem to indicate that residence in an institution may predispose to infection. The comparable low incidence in the small group of patients who were confined for long periods in the one *chronic* general hospital studied, however, points to the primary disease process as another major factor. Age was not a factor in this hospital, for the group was well distributed through all decades. The few psychiatric patients in the group were not in acute episodes, and this factor, to be discussed below, was negligible.

The part played by institutionalization requires

clarification by the study of additional institutions where non-tuberculous, non-psychiatric patients in a wide age range are confined for periods of two years or more.

Tuberculous vs. non-tuberculous

The reactions in the tuberculous group were more clear-cut, with fewer equivocal responses, than those in the non-tuberculous group. As will be seen in table 5, the incidence of 1 plus reactions is essentially the same in the two groups. If the strongly positive 2, 3, and 4 plus reactions in each group are combined, the total incidence of such reactions is 6.8 per cent in the tuberculous, and 6.1 per cent in the non-tuberculous group, a difference which probably is of no significance. No attempt was made to correlate the degree of reactivity with the stage of the primary disease; to evaluate this factor properly the same group of patients should be retested during convalescence. This may be delayed for years, however, and the factor of institutionalization itself then would enter. The striking finding in the non-tuberculous group, which was composed chiefly of psychiatric patients, is the higher incidence of questionably positive (±) reactions, and of positive reactions to both test and control (+T+C), and the occasional unexplained case in which there was no reaction to the test solution and a positive reaction to the control solution (−T+C), especially those which remained positive at twenty-four hours. These findings would seem to indicate that patients in psychiatric hospitals are not suitable controls. This possibility is discussed further below.

If psychiatric patients are excluded as controls, and if the factor of institutionalization is ignored (because of the findings in the *chronic* general hospital group as discussed above), the tuberculous group must be compared with the *total* general hospital group. In this group, which is nearly comparable in size to the group of tuberculous patients, the incidence of positive tests is less than half that in the tuberculous group, a finding previously reported (1).

Subsequent studies should include groups of non-tuberculous subjects without mental disorders who are confined to institutions for periods of time comparable to those required for the sanatorium treatment of tuberculosis. If a significantly higher incidence of positive skin tests to trichinella extract in persons with tuberculosis is confirmed, and if no true biologic cross-reaction between *Mycobacterium tuberculosis* and *Trichinella spiralis* is demonstrated in animals, several questions remain unanswered. Secondary infection by the tubercle bacillus is known to occur in tissue damaged by silicosis, sarcoid and Hodgkin's disease. Does the tubercle bacillus also have an affinity for tissue damaged by infection with trichinae? Experiments have shown that the spread of a dye in the skin is altered in patients with tuberculous infections (6). Do people with tuberculosis react more readily to other antigens of various sorts than do non-tuberculous individuals? Or does tuberculosis predispose the individual to infection by a smaller number of trichinae than are required to infect a person without the disease?

Psychiatric vs. non-psychiatric

The psychiatric as well as the tuberculous group was composed of patients who had been confined to an institution for months or years; the significance of this factor has been discussed above. If the tuberculous group is excluded, and the psychiatric group compared with the total general hospital group, the incidence of positive tests in psychiatric patients of all types and stages of disease is more than double than in the total general hospital control group.

It is known that the electrical resistance of the skin of *disturbed* mental patients may vary markedly from that of the same patients after recovery from the disturbed state (7). As far as we are aware, it has not been determined if the reactivity to biologic products, as opposed to physical agents, is also altered during the acute phase of a psychiatric disturbance. That this may be true is suggested by the findings in one mental institution (Morganton), where a perfect dietary control is furnished: the food is all prepared in a central kitchen from a single source of supply, and after it is cooked, distributed by steam carts to the various buildings. The incidence of positive tests in *convalescent* patients awaiting discharge and confined in one building *was comparable to that observed in non-psychiatric groups in acute or chronic general hospitals.* In contrast the incidence of positive tests in *disturbed* patients in another building who were on an identical diet *was approximately four times as great.* No attempt was made to evaluate the possible effect of the stage of the disease process on the figures for incidence as analyzed by duration of stay in an institution. The possibility of altered skin reactivity in various states of psychiatric disorders

must be determined with some biologic agent other than trichinella extract or tuberculin, since tuberculosis also is a disease which frequently is acquired during confinement in institutions.

SUMMARY

Of 1586 patients in institutions in North Carolina, 17.7 per cent gave positive skin reactions to trichinella antigen. The incidence of infections as determined by this method is in contrast to that found at autopsy in this area (2.8 per cent). This discrepancy has been attributed to the presence of light subclinical infections difficult to detect at autopsy; but it is possible that other factors, such as intercurrent disease, may have altered the incidence of positive skin tests in this study.

18.3 per cent of 674 tuberculous patients in sanatoriums and 17.2 per cent of 912 non-tuberculous patients in institutions (chiefly mental patients in psychiatric hospitals) gave positive reactions. In a previous study 6.9 per cent of 422 patients in general hospitals were found to give positive reactions.

The possibility is discussed that the type and stage of intercurrent disease may be a more important factor in reactivity to trichinella extract than is confinement in an institution, and that reactions to trichinella skin tests may be unreliable during a period of mental illness.

The incidence of positive skin tests was found to be greatest in patients confined in an institution for eighteen to twenty-nine months.

Race, sex, and residence in urban or rural areas are not significant factors. The incidence of positive skin tests decreases with advancing age.

The occurrence of delayed and persistent positive tests cannot be explained on the basis of very recent infection alone.

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METABOLIC ACCLIMATIZATION TO TROPICAL HEAT

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Present enforced activity of large military forces in tropical heat gives sharp emphasis to our need for a clear understanding of the influences such heat exerts. Just what does acclimatization, through the weeks and months of exposure, mean in terms of mental and physiologic processes? It is indeed unfortunate that several recent reports from government-sponsored war research projects have failed to present the matter in a balanced manner, tending to give the impression that such adaptation involves nothing more than the needed vasomotor adjustment.

Complete acclimatization to tropical heat has two quite distinct physiologic aspects. One of these is called into play during the first few days of heat exposure, while the other requires weeks for its appearance. When difficulty in heat dissipation is encountered by the unadapted subject, marked stress is placed upon the vasomotor mechanism for transport of waste heat from interior tissues to cutaneous surfaces. The normal person adapts fully to such vasomotor stress after 3 to 5 days of heat exposure, and he retains this phase of acclimatization quite well for months thereafter even though he receive only brief periods of re-exposure. Excellent studies by a number of laboratory and field investigators have established these points satisfactorily (1, 2, 3).

The second—and perhaps more important—phase of heat acclimatization is metabolic and of much slower accomplishment. It involves changes in combustion metabolism and in the functions of endocrine glands concerned therein (primarily the adrenals, thyroid, and pituitary, secondarily the gonads). Resting oxygen consumption declines rapidly during the last of the second and all of the third week of heat exposure (4), the blood pressure falls (5), gonadal activity is sharply restricted (6), and resistance to infection drops (7).

Mental functions seem similarly reduced, for medical aptitude tests given college students in middle temperate latitudes result in ratings 40% lower if the tests be given during July or August than if given in mid-winter (author's unpublished data). In an exhaustive study of examination records Huntington (8) found best mental function when outdoor temperatures were around 40°F., with the greatest handicap evident during summer heat.

Careful maze testing of litter-mate rats showed even more striking differences in learning ability (9). Those raised at 55°F. required 13 trials on the average to learn their way through the maze to food after a 24-hour fast; those kept for months at 75°F. required 23 trials, and those from a 90°F. environment 53 trials! Re-tested after a month's rest, the 55°F. rats showed complete retention of their earlier learning, the 75°F. ones had to relearn about half, while those adapted to tropical heat showed no evidence whatever of their earlier experience.

In seeking a metabolic basis for these slower adaptations to difficulty in heat loss, we investigated the dietary concentrations of the various B-vitamins needed to support optimal growth in weanling rats kept at 68°F. and at 90–91°F. Thiamine and choline were the only ones for which distinct differences in requirement were found, best growth being attained in the heat when the dietary thiamine was double the 68°F. optimum and the choline raised from 0.75 gm./kg. to 5 gm./kg. These changes in the two vitamins, together with a slight increase in the protein content or the addition of 0.2% cystine, resulted in the same growth rate and physical characteristics at 91°F. as at 68°F.—but on only 70% as much food. Phagocytes from the blood and peritoneum of hot room animals exhibited just as great activity as did those from animals kept at 68°F., provided the above-mentioned dietary corrections were made (10). Such corrections have also been found to render animals better able to withstand excessive heat.

Several investigators, unfortunately testing these findings without due regard to the time required for metabolic adaptation, failed to verify any such increased vitamin requirements for

human subjects exposed to tropical heat. In every case, however, their periods of heat exposure were too short to have been expected to be effective. Keys et al. (1) failed to find any benefit from vitamin supplementation in normal subjects *exposed to heat for one week or less* in Minneapolis, and on this basis decided that tropical needs were not different from those of temperate coolness. In Boston Johnson (11) found no metabolic or work differences in normal subjects *exposed to high temperatures only for the few hours of observation in the laboratory each day* and from this concluded that nutritional standards were the same in tropical and temperate climates. He further bolstered his arguments by the statement—unsupported by published data—that his subjects reacted the same in summer as in winter, *presuming to liken Boston's mild mid-day summer warmth to the continuous moist heat of tropical regions.* Holt (12) correlated urinary thiamine output with daily temperature changes and found no apparent rise in thiamine requirement accompanying a few days of heat in Baltimore. From such a study he claimed that our animal findings were not applicable to man, entirely disregarding the time interval needed for such metabolic adaptation.

Still another instance of disregard for the time factor is to be found in a recent article by Taylor (13), for he cites human tests covering only a few days as disproving any effect of environmental temperature upon vitamin needs. He further suggests that our rat studies were of doubtful significance for man because Herrington (14) had found rectal temperatures of rats to be 2.5°F. higher in an environment of 90°F. than in one of 68°F.; *but Herrington's rats were exposed to the heat for only a few hours of observation* and were given no chance for metabolic adaptation. We have found the rectal temperature of rats and mice adapted for weeks at 90°F. to be insignificantly different (usually less than 0.5°F.) from those of animals similarly adapted at 68°F.

In presenting our evidence that the concentration of dietary thiamine needed for optimal rat growth was twice as high at 91°F. as at 68°F., our published data showed that these differences were not present during the first week of exposure to heat or cold,—that they began in the second and became fully developed in the third week. Many repetitions of this experiment have provided uniform verification. Recent tests, not yet reported in detail by us, have also shown that addition of 0.5% sulfaguanidine to the diets in no way alters these differences in requirement from the third week on; therefore they cannot be based upon variations in vitamin synthesis by intestinal bacteria.

We have recently compiled all our rat growth data at different dietary concentrations of choline, with the following results:

	AT 68°F.				
Dietary choline (gm./kg.)	0.0	0.4	0.75	1.5	3.0
Wt. gain in gms. 2nd, 3rd, & 4th weeks on diet	85.00 ±4.77	80.72 ±2.09	86.00 ±2.35	96.00 ±4.20	90.00 ±5.56
	AT 90–91°F. AND 60–70% REL. HUM.				
Dietary choline (gm./kg.)	0.0	0.4	1.5	3.0	5.0
Wt. gain in gms. 2nd, 3rd, & 4th weeks on diet	37.86 ±5.25	56.11 ±2.62	61.36 ±2.09	71.88 ±2.43	83.39 ±1.07

Here is presented the surprising finding that growth in the heat is directly proportional to the *choline content of the diet up to the 5 gm./kg.* level. At 68°F., on the other hand, it seems to make little difference whether choline be present or absent after the danger period for hemorrhagic nephritis is passed. Choline (or other supplier of labile methyl) must thus be regarded as a particularly important nutritional factor in tropical heat.

Rats on diets containing the various choline concentrations show little difference in growth rate during the first week in heat or cold; and during the second week the incidence of acute hemorrhagic nephritis is approximately the same in rats at 68°F. as at 90°F. on choline-free diets. From the third week onward, however, the choline influence over growth becomes clear-cut and definite in the heat. Here again is evidence of the delay in metabolic acclimatization to tropical heat, with maximal effects appearing during the third week of exposure.

Addition of 0.5% sulfaguanidine to the diets fails to alter this choline-growth relationship. Normal growth in the cold still goes on with little or no choline, while in the heat 5 gm./kg. remains optimal. Intestinal synthesis seems here again to play an insignificant part.

The vasomotor adjustment taking place in the first few days of heat exposure is an important

phase of acclimatization, one which is necessary in the prevention of such acute emergencies as heat stroke and heat exhaustion. However, it seems in no way related to the slower metabolic phase of adaptation which requires weeks of exposure and involves profound changes in cellular combustion and the endocrine system. Workers and writers in the field of acclimatization should keep clearly in mind these time differences in the two phases of body response to heat and cold.

CONCLUSIONS

Metabolic acclimatization to tropical heat takes place largely in the third week of continuous exposure. When fully developed, it involves the need for a doubling of dietary thiamine per unit of food and a many-fold increase in choline. In the heat choline (or other supplier of labile methyl groups) becomes a nutritional factor of great importance. After the danger period for acute hemorrhagic nephritis has passed, growth rate in rats is strongly dependent upon the concentration of dietary choline in the heat but not in the cold.

Added to these changes in vitamin needs for optimal nutrition, acclimatization to tropical heat involves endocrine alterations which markedly affect the subject's ability to work and think. There is no conclusive evidence yet as to whether proper vitamin supplementation would prevent or lessen the metabolic effects of tropical heat on man, although preliminary tests indicate that such may be the case.

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WARTIME TROPICAL MEDICINE ACTIVITIES OF THE NATIONAL RESEARCH COUNCIL

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The wartime activities in tropical medicine of the National Research Council began in May, 1940, with the appointment of the Subcommittee on Tropical Diseases in the Division of Medical Sciences. The original Subcommittee consisted of Dr. W. A. Sawyer, Chairman, Dr. M. F. Boyd, Dr. E. H. Hume, Dr. T. T. Mackie, Dr. R. B. Watson, and Dr. Henry E. Meleney. Since that time a number of changes and additions have been made in the membership of the Subcommittee. Dr. Meleney became Chairman in January, 1941, and the other members at the present time are Dr. M. F. Boyd, Dr. H. W. Brown, Dr. W. W. Cort, Dr. K. F. Meyer, Dr. G. K. Strode, Dr. W. H. Taliaferro, Dr. H. B. Van Dyke, Dr. R. B. Watson and Dr. A. A. Weech.

The functions of the Subcommittee have been to prepare directives for the treatment and prevention of tropical diseases, to make recommendations to the armed forces, and to initiate and supervise research.

The first directive on the treatment and prevention of tropical diseases was issued by the Surgeon General of the Army as Circular Letter No. 56, dated 9 June 1941. A revised and expanded edition was issued as Circular Letter No. 33 on 2 February 1943. These directives were also used as a basis for the development of a manual on tropical diseases issued by the Bureau of Medicine and Surgery of the Navy.

The great importance of malaria led to the formation of a Conference Group on Malaria Research which held its first meeting under the Chairmanship of Dr. L. T. Coggeshall on 8 July 1941. This group was at first subsidiary to the Subcommittee on Tropical Diseases, but later became an independent Subcommittee for the Coordination of Malarial Studies. Its main interest was a search for better chemotherapeutic agents, and it included four panels dealing respectively with chemistry, synthesis, pharmacology and clinical testing. This Subcommittee was in turn replaced in November, 1943, by a Coordinating Board for Malarial Studies under the Chairmanship of Dr. Robert Loeb of Columbia University and containing members of the previous panels and representatives of the Army, Navy, Public Health Service, and Office of Scientific Research and Development.

In August, 1941, funds became available for the support of research under the Office of Scientific Research and Development. The Subcommittee on Tropical Diseases has sponsored up to the present time forty-five projects which have received contracts under this arrangement. The fields covered by these research projects are malaria, insect repellents and insecticides, bacillary dysentery, plague, cholera, water purification, parasites in sewage, filariasis, schistosomiasis, leishmaniasis, and amoebiasis.

The research on malaria has dealt with chemotherapy, immunology and biological problems. The first productive work in chemotherapy was the study of quinacrine (atabrine) with special reference to blood levels and toxic reactions. Blood level studies showed the necessity of administering large doses during the first twenty-four hours in order to obtain rapid response to treatment. The study of toxic reactions showed that these were relatively unimportant and led to a more rational and effective method of using the drug as a suppressive agent. Circular Letter No. 153 from the Office of the Surgeon General of the Army dated 19 August 1943, and War Department Medical Technical Bulletin No. 65 dated 3 July 1944, outlining the approved therapeutic and suppressive use of quinacrine resulted from this research. The search for new chemotherapeutic agents against malaria has involved the cooperation of many chemists and the testing of compounds against bird malaria by a number of laboratories. The final testing of promising com-

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pounds in man has been performed mainly on cases of therapeutic malaria. The results of this work are still not available for presentation for reasons of military security.

Work on the intermediate stages of the malaria parasites between the sporozoite and erythrocytic forms has demonstrated practically the complete history of this so-called cryptozoic stage in certain bird malaria parasites (1), and similar work is in progress with the parasites of monkey and man. In the immunological field considerable study has been made of the complement-fixation reaction, and the interesting fact has recently been reported that the stromata of normal human erythrocytes can be used as an antigen for this test in man (2).

Perhaps the most productive research has been in the field of insect repellents and insecticides. This has been due primarily to vigorous and extensive studies conducted by the U. S. Department of Agriculture under a transfer of funds from the Office of Scientific Research and Development. Three repellents have been discovered which individually or in combination give protection for two hours or more against most species of mosquitoes. The development of an effective lousicide containing pyrethrins and other ingredients had already been developed when the now well-known DDT was discovered and became the number one insecticide for general use. The determination of the extent of the usefulness of this substance not only against disease-carrying arthropods, but against agricultural pests as well, will require a long period of experimentation.

Promising observations have been made on protecting the interior of habitations from the invasion of Phlebotomus by spraying around entrances. This procedure may be valuable in the prevention of diseases transmitted by these flies. Observations of interest have also been made on the mode of action of insecticides and repellents.

In the field of bacillary dysentery valuable contributions have been made with reference to the choice of preparation and mode of administration of the sulfonamides. Studies of the toxins and antigens of the dysentery bacilli have also been made in the hope of developing an effective vaccine. Field trials of vaccines are now being made in this country among civilian groups.

Antigenic studies of the cholera vibrio have yielded the interesting observation that the antigenic and toxic substance is probably a phospholipid which causes increased permeability of the wall of the intestine to fluids.

New methods of filtration and chemical treatment of water to kill the cysts of *Endamoeba histolytica* as well as pathogenic bacteria have been developed. Supervision of this research has been transferred to the Committee on Sanitary Engineering.

The most recent research projects developed by the subcommittee have been in connection with the chemotherapy of filariasis, schistosomiasis, leishmaniasis, and amoebiasis. A Chemotherapy Center has been established in the Division of Medical Sciences to coordinate research on these subjects, and to furnish new chemotherapeutic agents for testing. This work is not yet advanced to the point of obtaining results. Related to this field, there is also a study of domestic snails as possible intermediate hosts of the schistosomes of man, and of domestic species of Phlebotomus as possible vectors of leishmaniasis.

The Subcommittee on Tropical Diseases has also been active in promoting instruction in Tropical medicine both in the armed forces and in medical schools. In May, 1941, the Subcommittee recommended that medical officers of the Army and Navy should be given special training in tropical medicine. Members of the committee cooperated in developing a course at the Army Medical School, and Dr. T. T. Mackie entered the Army Medical Corps and became Executive Officer of that course. The course was soon lengthened from four weeks to eight weeks, and has been repeated almost continuously since its inception in the autumn of 1941. At one time, classes of 200 medical officers were accommodated in this course. In addition, it has been open to instructors from medical schools and to members of other governmental agencies. The Navy also has developed excellent training in tropical medicine for both officers and enlisted personnel.

The Subcommittee also approved and sponsored appropriations by the John and Mary R. Markle Foundation to the Division of Medical Sciences for improving the teaching of tropical medicine in medical schools. Two appropriations were made, one to support visiting lecturers to medical schools, the other to support the preparation and distribution of maps and epidemiological material on tropical diseases in the possession of the Office of the Surgeon General of the Army. The visiting lectureships have been of considerable value in

presenting specific problems to medical students and interns, and in stimulating better instruction by medical schools. Fourteen maps showing the distribution of tropical diseases were distributed to medical schools in large and small sizes, and as lantern slides. The first volume of the epidemiological material has recently been published under the title "Global Epidemiology", dealing with India and the Far East, and the Pacific area. Two copies of this volume are being furnished to each medical school.

Throughout this entire period of activity in tropical medicine, the National Research Council has had the heartiest cooperation from the medical services of the Army and Navy, from the United States Public Health Service, and from innumerable individuals and institutions throughout the country. There has also been excellent liaison with the representatives of Allied Governments. This spirit of cooperation has undoubtedly contributed considerably to the maintenance of a high level of health among our armed forces operating in the tropics.

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A METHOD FOR COUNTING THE MICROFILARIAE OF LITOMOSOIDES CARINII OF THE COTTON RAT[1]

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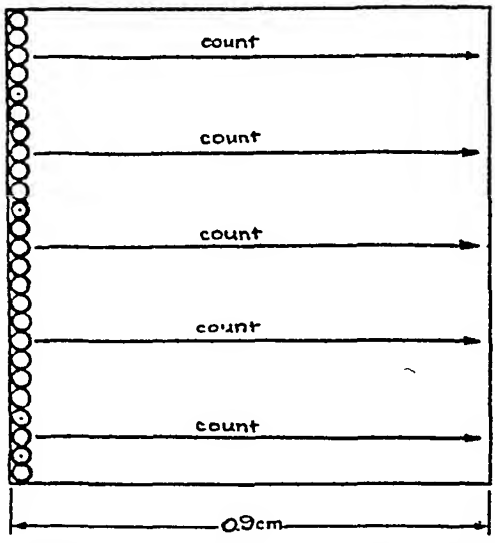
Recent studies indicate that the filariid *Litomosoides carinii* of the cotton rat, *Sigmodon hispidus*, is a useful parasite for the study of new chemotherapeutic agents. To ascertain the effects of experimental therapy the reduction in microfilariae in the blood may be followed and later the effect on the adult worms in the pleural cavity observed at autopsy. A method for counting the microfilariae should be accurate, rapid and, if possible, permit a permanent record for review if necessary. After trying various methods the following technic was found to be highly satisfactory.



1. Mark off a square 0.9 of a centimeter on a side with a diamond pencil upon a glass slide.
2. Draw blood from tail of cotton rat to the 0.5 mark on red blood cell hemocytometer pipette.
3. Blow out blood onto marked square and spread evenly with fine wire used to clean syringe needles and allow to dry.
4. Stain for 45 minutes with Giemsa. Giemsa concentrated stain 1 cc. to 50 cc. buffered distilled water pH 7.2.
5. Wash 15 minutes in buffered distilled water and allow to dry.
6. Under high dry power and 10× eyepiece beginning with the third row count the microfilariae in every 5th row of microscope objective fields. Multiply the microfilariae by 5 to ascertain total microfilariae on the slide.

Microfilarial counts of *L. carinii* often run from 200,000 to 1,000,000 per cc. of rat's blood, hence examining and counting the blood of the whole square is very time-consuming. In order to save time, we therefore made a careful study to ascertain whether or not counts of every 5th line of the square when multiplied by five would give a sufficiently accurate approximation of the total microfilariae on the whole slide. Two squares, A and B, were filled with blood to ascertain whether or not duplicate smears taken at the same time were necessary for accuracy.

Table 1 gives the data on microfilarial counts when the above method was used. Each individual row count was kept separate and the total microfilariae on Square A was 336 and Square B 321. When every 5th row was counted and added together and the result multiplied by 5 the total microfilariae on Square A varied from 290 to 400 and on Square B from 300 to 355. These counts were within the range of accuracy of the blood sampling technic and statistical significance. Fifteen slides, each with two blood specimens, were counted in a check on the accuracy of the method. An example of a high count follows (table 2).

When the microfilarial count drops as low as 50 per slide (approximately 3.2 per cu. mm.) it is advantageous to count all of the microfilariae on the whole slide as the counting of only one fifth of the slide may be statistically inaccurate (table 3).

The hemocytometer pipettes used were found to vary somewhat in the amount of fluid necessary to fill them to the 0.5 mark. They were calibrated by filling up to the 0.5 mark with mercury which was then weighed and the volume displaced calculated. Three hemocytometer pipettes purchased from Will Corporation had volumes up to

[1] This study was made possible through financial support of the John and Mary R. Markle Foundation. We wish to acknowledge the assistance of Dr. John W. Fertig who analyzed our data statistically.

the 0.5 mark of 3.2 cu. mm., 3.2 cu. mm. and 3.1 cu. mm. One purchased from the Bausch and Lomb Company had a volume of 3.7 cu. mm. If the same pipette is used for all the blood studies in an experiment its actual volume is not important. If a number of pipettes are used, however, it is essential that their actual volume be ascertained. By calibrating the pipette in cubic millimeters all counts can then be calculated in terms of microfilariae per cc. of blood.

TABLE 1

ROW	NUMBER OF MICROFILARIAE	
	Square A	Square B
1	14	13
2	13	17
3	17	15
4	8	13
5	18	8
6	26	11
7	15	10
8	16	10
9	19	6
10	9	9
11	28	14
12	9	7
13	13	17
14	10	15
15	13	17
16	5	10
17	18	15
18	15	11
19	13	13
20	8	14
21	7	17
22	9	15
23	9	18
24	8	14
25	16	12
Total microfilariae on square.....	336	321

Total number of microfilariae on square when every 5th row was counted and multiplied by 5

	SQUARE A		SQUARE B	
		Dif.		Dif.
Every 5th row beginning with				
1 =	400	+64	325	+4
2 =	320	−16	320	−1
3 =	350	+14	355	+34
4 =	290	−46	305	−16
5 =	320	−16	300	−21
	5 | 1680 / 336	5 | 156 / 31.2	5 | 1605 / 321	5 | 76 / 15.2

A square of 0.9 centimeter usually provides approximately 25 rows the diameter of the high power objective, and is the reason that this size square was chosen.

TABLE 2

Total microfilariae counted on 0.9 square centimeter = 1438. Estimated microfilariae when every 5th row was counted and the results multiplied by 5

	DIFFERENCE
Beginning with row number 1—total microfilariae = 1425	−13
Beginning with row number 2—total microfilariae = 1475	+37
Beginning with row number 3—total microfilariae = 1460	+22
Beginning with row number 4—total microfilariae = 1455	+17
Beginning with row number 5—total microfilariae = 1375	−63

TABLE 3

Total microfilariae counted on 0.9 square centimeter = 40. Estimated microfilariae when every 5th row was counted and the results multiplied by 5

	DIFFERENCE
Beginning with row number 1—total microfilariae = 50	+10
Beginning with row number 2—total microfilariae = 25	−15
Beginning with row number 3—total microfilariae = 50	+10
Beginning with row number 4—total microfilariae = 40	0
Beginning with row number 5—total microfilariae = 40	0

A possible source of error in the method described is the loss of microfilariae during the staining and washing of the slides. To ascertain the extent of the loss, if any, of microfilariae from the slides the staining solution and wash water used on several heavily infected smears were centrifuged and examined for microfilariae but none were found.

Occasionally a small amount of blood may be smeared outside of the marked square but it has been our experience that no more than 1.6% of the larvae are found outside the square in carefully prepared smears.

During warm or hot weather we have found that the cotton rats' blood coagulates rather quickly in the pipette and it is very difficult to make a good blood smear. By cooling the pipette in a refrigerator before drawing the blood this rapid coagulation is prevented and satisfactory smears can be made.

Bell and Brown[3] have shown, using the above technic, that although there is no regular periodicity in the appearance of the microfilariae of *L. carinii* that there is a considerable variation in circulating microfilariae from hour to hour and from day to day.

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BOOK REVIEWS

The Argasidae of North America, Central America and Cuba. By R. A. Cooley and Glen M. Kohls. 3 p. l., 152 p., 57 fig., 14 pl. (The American Midland Naturalist Monograph. No. 1.) The University Press, Notre Dame, Ind., 1944.

This compact volume, constituting Monograph No. 1 of the American Midland Naturalist, contains a wealth of information about the soft ticks of North and Central America. It fills a long-standing need for an adequate and up-to-date taxonomic treatise on this important group of arthropods in the New World. The authors, who are eminently qualified for the difficult task involved, have done an admirable piece of work which may well serve as a model in the exacting and fundamental field of taxonomy. Clarity, conciseness, and completeness in both text and illustrations are evidence of thorough digestion and evaluation of the many details that must be considered in the preparation of useful descriptions of a large series of closely similar species.

The authors recognize, within the area covered, four genera and twenty-four species in the family Argasidae. The genotypes are fully described, including the Old World *Ornithodoros savignyi*. For each species the following information is given: a list of synonyms; a full description of the adult stage, when known; shorter descriptions of larval and nymphal stages, as far as possible and necessary; line drawings of significant structural details; photographs of dorsal and ventral views of representative specimens; host data; biological notes; distribution records. For the important and more common species detailed tables of collection data and spot-distribution maps are appended. Differences between closely similar species are indicated, and *Ornithodoros turicata* and *O. parkeri* are compared at length in all stages. The principal morphological descriptions follow a uniform pattern, with separate paragraphs given to each structure. The line drawings accompany the descriptions, while most of the photographs appear as plates at the end of the text.

Keys to genera and species are provided to aid identification of adult and nymphal specimens. The utility of the treatise is much enhanced by the inclusion of an illustrated glossary defining precisely the descriptive terms employed, thus avoiding the ambiguity which so often lessens the value of keys and descriptions for non-specialists. The authors are to be commended upon thus systematizing the terminology for this group, as the senior author has also done for the Ixodidae in an earlier publication (National Inst. of Health Bull., No. 171). This should provide a firm basis for uniform precision in future work on the taxonomy of ticks. An extensive bibliography and detailed index are also included.

Parasitologists and medical entomologists in general will find of especial interest the summary of the medical and veterinary importance of the Argasidae, the descriptions of methods for study and rearing, the classified list of hosts, and the geographical list of species. Five species (*Ornithodoros hermsi, turicata, parkeri, talaje,* and *rudis*) are proved vectors of relapsing fever in the western United States, Central America, and South America. *O. parkeri* and *hermsi* (and, according to subsequent reports, *O. nicollei*) can transmit Rocky Mountain spotted fever, and *O. hermsi* American Q fever. *O. coriaceus* and *stageri* inflict painful bites on man. *Argas persicus* is a poultry pest which acts as a vector of avian spirochaetosis and possibly of fowl paralysis, and the spinose ear tick, *Otobius megnini*, is an important pest of cattle which has occasionally been found also in the ears of man. Reptiles, birds, bats, carnivores, ungulates, rodents, and primates are included in the host list, eleven species of soft ticks having been recorded from man. Nineteen species occur in the United States.

Teachers and research workers will regret that no mention is made of the internal anatomy of soft ticks. A brief summary of this subject, or at least references to it, would have been a valuable addition.

The high quality of the contents of the volume is matched by the excellence of its physical make-up. It is well printed on good paper, durably bound, and has only a few minor typographical errors.

The real value and adequacy of a work of this kind for the accurate identification of specimens can be determined only by actual use of the keys and descriptions. There is every indication that this monograph will stand the test of time and use, and it is to be hoped that the authors and other competent specialists will continue to build well upon the good foundation it supplies.

Albert Miller.

Laboratory Methods of the United States Army, 5th Edition. Edited by James Stevens Simmons, B.S., M.D., D.P.H., Ph.D., Sc.D. (Hon.), Brigadier General, U. S. Army, Chief, Preventive Medicine Service, Office of The Surgeon General, U. S. Army, and Cleon J. Gentzkow, M.D., Ph.D., Colonel, M.C., U. S. Army, Commanding Officer, Deshon General Hospital. Lea & Febiger, Philadelphia, Pa., 1944. Illustrated. Pp. 1–14, 15–823.

This revised edition contains 103 engravings and 8 color plates, with twenty-five contributors from both the U. S. Army and civilian institutions. Advice and assistance was given by many from the Army, U. S. Public Health Service and prominent civilians.

The compilation of contents is divided into XI parts, and covers: Clinical Pathology; Chemistry; Mycology; Bacteriology; Rickettsiae and Filtrable Viruses; Protozoology; Helminthology; Entomology; Pathology;

Special Veterinary Laboratory Methods, and Statistical Methods.

With so many subjects it is condensed to the essential recognized technical procedures. The printing and paper are excellent. The binding is poor. It contains much valuable and useful information, and is a cross-section of thought from many parts of the country. Necessity demands that for all students no one book suffices.

HARVEY R. LIVESAY.

Malaria: Its Diagnosis, Treatment and Prophylaxis. By WILLIAM H. BISPHAM, Colonel, U. S. Army, Retired. Pp. 1–183 with V plates, four of which are colored. The Williams & Wilkins Company, Baltimore, Md. 1944.

This book has been written to give the physician a knowledge of the clinical features of malaria. In keeping with this expressed purpose the author has considered such subjects as Etiology, Epidemiology, and Pathology only briefly. There is a more detailed discussion of "Symptomatology" and "Treatment."

A useful addition to a book of this type would be a concise chart of the differential characteristics of the various species of the Plasmodia that produce malaria in man. Such a chart would be of definite assistance to the physician in making a correct diagnosis of malaria.

The protean clinical manifestations of the three diseases grouped under the term malaria are completely considered. The various types of pernicious malaria are classified according to symptoms and are discussed.

Drugs and treatment methods are discussed. The physician who has not had experience with the treatment of malaria would welcome a definite recommended dosage regime of the various drugs employed. The newer dosage of atabrine, using a large initial dosage and smaller maintenance doses, appears in the chapter on "Prevention and Treatment of Malaria in West Africa," by DR. L. T. COGGESHALL.

In the discussion of intravenous quinine there appears to be a miscalculation, as the injection of 8 ounces (240 cc.) of a solution containing 25 grains in 10 ounces (300 cc.) will give 19.2 grains, not 10 grains as stated.

No mention is made of the effective intramuscular administration of atabrine.

The chapter "Prevention and Treatment of Malaria in West Africa" by Dr. L. T. Coggeshall, contains much useful information about malaria in general.

This book should be useful to the physician in giving him a more thorough understanding of the clinical features of malaria.

FRATIS L. DUFF.

Fundamentals of Internal Medicine, 2d ed. By WALLACE MASON YATER. B. Appleton Century Company. 1944. Pp. I–XLI, 1–1204.

One can scarcely picture a more ambitious undertaking nor one which comes closer to realization than that undertaken by the author in this work. In the space of 1,169 pages he not only covers practically every disease known to internal medicine but has space in which to include a well rounded discussion of psychoses and psychoneurosis.

It is also a surprise but a pleasant one, to find a section on the diseases of the skin. The internist may believe that he may choose the limitations of his knowledge but it is otherwise with the patient. The patient does not expect to be sent to a dermatologist for every skin lesion which seems simple to him. This section gives all of the common skin diseases which one is likely to encounter together with well chosen illustrations.

Although the book is supposed to deal only with fundamentals almost any section covers the subject very thoroughly and in some instances to a surprising degree. In a few instances there are omissions of valuable diagnostic measures such as the complement fixation test in the rickettsial diseases but in general the book is well abreast of the current medical literature.

The section on dietetics and the list of diseases given according to abnormal findings in the laboratory, provides a quick reference. The work is quite in keeping with the growing tendency to stick to the facts in an undertaking of this type and to leave the extrapolations of theory and elegance to special monographs.

To prepare a work upon the fundamentals of diseases requires an extremely broad outlook and background of medicine. The work itself testifies to these qualifications in the author. The book will prove to be extremely useful to anyone who keeps it handy.

HENRY M. WINANS.

BAILEY K. ASHFORD AWARD IN TROPICAL MEDICINE

At the annual meeting of the American Society of Tropical Medicine in Memphis, the Bailey K. Ashford Award in Tropical Medicine was established by Eli Lilly & Company. The award will be $1,000.00 and a bronze medal suitably engraved. An additional amount of $150.00 or as much thereof as may be necessary is available toward traveling expenses for the recipient of the award.

Conditions of the Award

As adopted by the Council November 10, 1941

1. The Bailey K. Ashford Award will be given annually in recognition of demonstrated research in the field of tropical medicine, taking into consideration independence of thought and originality.

2. The investigator must be a citizen of the United States of America and less than 35 years of age on January 1 of the year in which the award is made. The recipient must not be associated with a commercial laboratory and need not be a member of the American Society of Tropical Medicine.

3. Members of the American Society of Tropical Medicine shall be requested to submit to the Secretary of the Society (in triplicate) the name of a proposed recipient with full information concerning the personality, training and a statement of the research work for which the award is to be made. Such nominations must be in the hands of the Secretary at least six months before the date of the award and will be forwarded to the Committee of Award immediately after that date.

4. The Committee of Award shall have complete freedom, within the limitation of the above conditions, in selection of the recipient but shall give due consideration to nominations submitted by members of the Society. The selection of the recipient shall be reported to the Secretary of the Society at least four months before the date of the award.

5. The recipient will be given opportunity to present a short review of his work at the meeting at which the award is made.

6. The Committee of Award shall consist of the President of the Society ex officio and three members of the Society, each to serve for a period of six years and one to be elected every other year at the annual meeting by the Council; except that the original committee be elected at once by the Council on nomination of the President, one member to be elected for six years, one for four years and one for two years.

7. The name of the recipient and suitable recognition to the donors are to appear on the program of the scientific meetings of the Society.

SOME EPIDEMIOLOGICAL ASPECTS OF INFECTIOUS HEPATITIS IN THE U. S. ARMY[1]

DOUGLASS W. WALKER[2]

Received for publication December 8, 1944

Infectious hepatitis is a well known affliction of armies in the field, and is particularly prevalent during times of war.

In a recent paper by Lucké, reference is made to epidemics of this disease in foreign armies in the Franco-Prussian and South African Wars and in World War I. In the American Civil War there were over 50,000 cases and 231 deaths among Federal troops, and a small epidemic occurred in our Army of Occupation in 1919.

Infectious hepatitis has not been confined to the military, however. During the past century and a half civilian outbreaks have been noted in England and Finland, and British investigators have studied the epidemiology extensively. In this country the disease has been reported by Blumer and others.

Yet, despite considerable study, the nature of the etiologic agent and the manner of its transmission have remained obscure. It was realized that the relatively infrequent epidemic outbreaks stemmed from one or several cases which multiplied over weeks or months to reach a peak in the fall of the year, and then quickly subsided. The incubation period appeared to be about 30 days. The causative agent, possibly a virus, was thought to be transmitted by respiratory tract discharges from an infected individual. The mortality was only 0.2% to 0.4%, and surprisingly constant. Death was usually due to yellow atrophy of the liver.

Another form of jaundice with the same symptomatology and clinical course had also been recognized following injections of human blood, plasma or serum. This type of hepatitis differed from the naturally occurring illness in two respects: a prolonged incubation period of 2 to 4 months and a low degree of communicability. Although several articles had been published concerning "homologous serum jaundice," it received relatively little attention in this country until an extensive outbreak in the United States Army in 1942 which was associated with the administration of certain lots of serum-containing yellow fever vaccine. This epidemic gave a powerful impetus to and afforded a unique opportunity for study of this type jaundice by American investigators. The subsequent natural occurrence of epidemic hepatitis in the Army in certain overseas areas re-emphasized the importance of this infection in military medicine, and provided the opportunity for its concerted study also.

Certain epidemiological aspects of outbreaks of these two types of jaundice are described briefly as follows:

I. THE OUTBREAK OF JAUNDICE IN THE ARMY IN 1942 (AN EXAMPLE OF HOMOLOGOUS SERUM JAUNDICE)

Early in 1942 an increased incidence of jaundice in the Army in the United States was noted by the Preventive Medicine Service of The Surgeon General's Office. By March it was evident that a widespread epidemic was imminent, and immediate steps were taken to prevent its further spread. Jaundice was made reportable by radio from all commands, and teams of investigators were organized and sent into the field. In addition, a report form was required on all cases and forwarded to The Surgeon General's Office for detailed analysis.

It was soon apparent the epidemic was not limited to the continental United States, but was occurring simultaneously in troops in such widely separated regions as Hawaii, the Southwest Pacific, Alaska, Iceland and England. However, despite the magnitude of the outbreak and its wide dispersion, The Surgeon General was able to take action to bring it under control within a short time. Data submitted by a team of the Army Epidemiological Board seemed conclusively to exclude toxic agents or contacts with infected civilians as causes, but did establish statistically a causal relationship between the administration of certain lots of yellow fever vaccine 2 to 3 months previously and the cases of jaundice.

On the basis of this information, The Surgeon

[1] Presented at the Annual Meeting of the American Society of Tropical Medicine in St. Louis, Mo., November 14, 1944.

[2] Major, Medical Corps, A.U.S., Executive Officer, Preventive Medicine Service, Office of the Surgeon General, Washington, D. C.

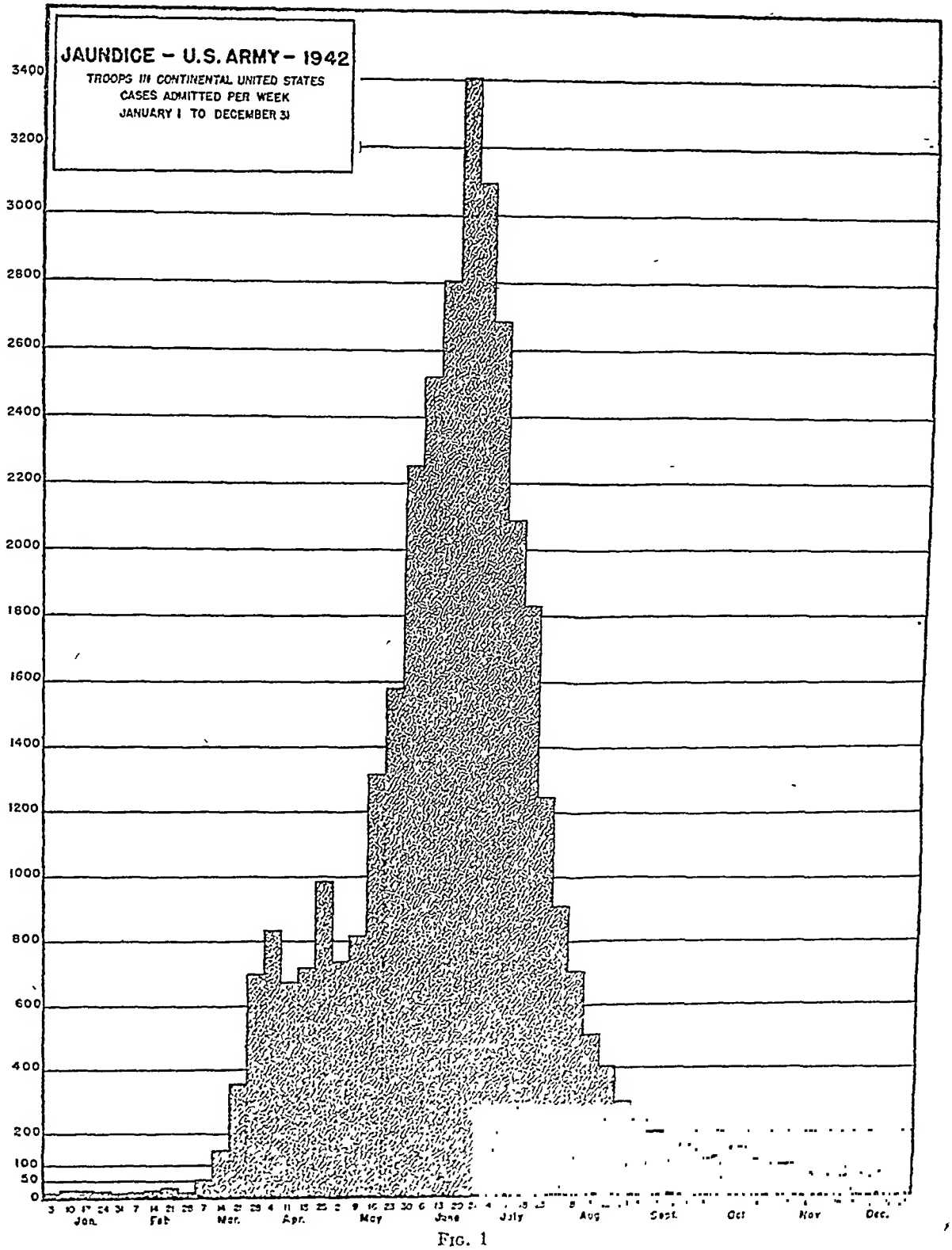

FIG. 1

General on April 15, 1942 discontinued the administration of serum-containing yellow fever vaccine.

At that time it seemed possible an icterogenic agent, perhaps the unknown cause of infectious hepatitis, had been introduced into the vaccine in human serum used in its preparation. The

subsequent development of the epidemic in the Army, and later epidemiological and laboratory studies, adequately supported the wisdom of The Surgeon General's decision. A serum-free vaccine was substituted, and no jaundice, proven to be associated with yellow fever vaccination, has since developed in recipients of the serum-free preparation.

Some of the main points of interest in this outbreak are graphically illustrated in the following figures:

Figure 1 shows the reported admissions for jaundice in troops in the continental United States during 1942. There is an ill-defined primary peak, followed approximately 2 months later by a second, higher peak, which falls off rather sharply to the usual baseline incidence for the Army. An epidemic with two such peaks is not unusual, and might indicate a primary and secondary wave of a highly contagious disease. But this outbreak was "out of season," and the explanation for this type of curve lies in the manner in which the Army's vaccination program against yellow fever was carried out. During the latter part of 1941 all Air Corps personnel and Ground Forces troops alerted for overseas duty were inoculated with yellow fever vaccine. The vaccine administered at this time included three highly icterogenic lots. Between January 20 and April 15, 1942, all other personnel then in the Army were vaccinated. Included in the lots of vaccine used at this time were four more highly icterogenic lots, most of which were used during February and early March. Since the incubation period for homologous serum jaundice is from 2 to 4 months, it immediately becomes apparent that the first peak resulted from the use of icterogenic lots in late 1941. Similarly, the later administration of additional icterogenic lots to a much greater number of troops gave rise to a second and higher peak.

Figure 2 shows admissions for jaundice in troops overseas. The first peak represents jaundice in troops in Hawaii; the second represents chiefly jaundice in England, Ireland and Iceland; while the third sharp rise was an isolated outbreak at a post in Alaska where inoculations against yellow fever with an icterogenic lot of the vaccine were carried out in May of 1942, one month after the rest of the Army had ceased using this product.

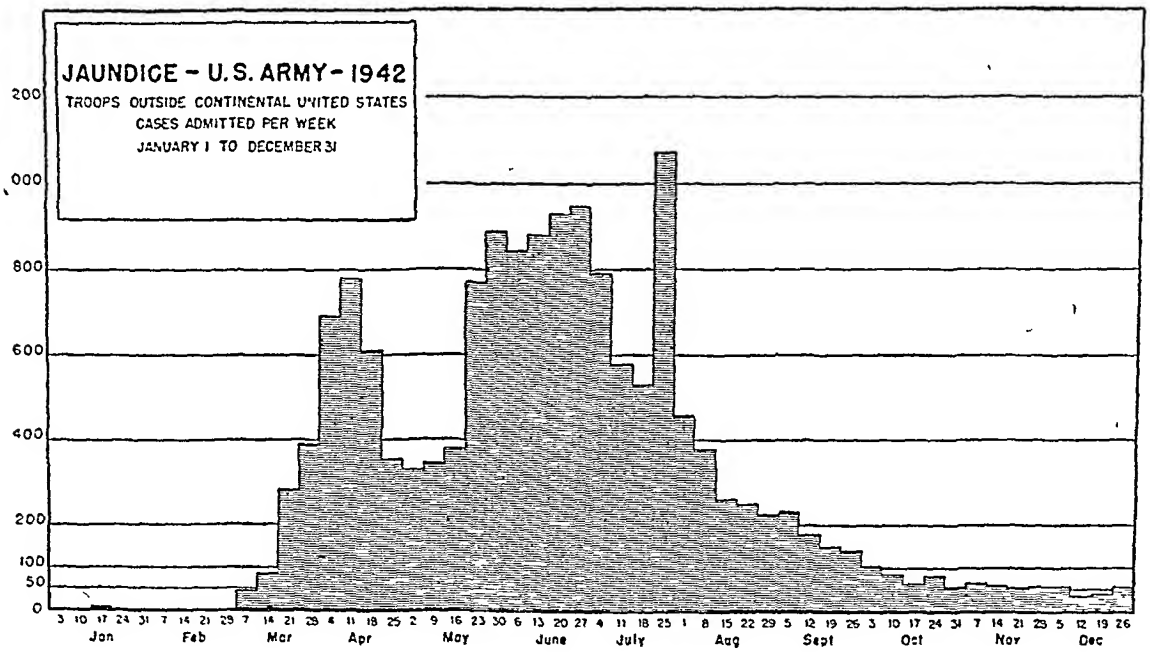

FIG. 2

Figure 3 combines the two previous graphs to give a composite curve of the admissions from jaundice in the entire Army in 1942. A total of 51,337 cases were reported. The two distinct peaks are readily noticeable in this figure.

Figure 4 shows admissions from jaundice by lot numbers of yellow fever vaccine. It is fairly easy to pick out those lots which were highly icterogenic. They were numbered 331, 334, 335, 338, 367,

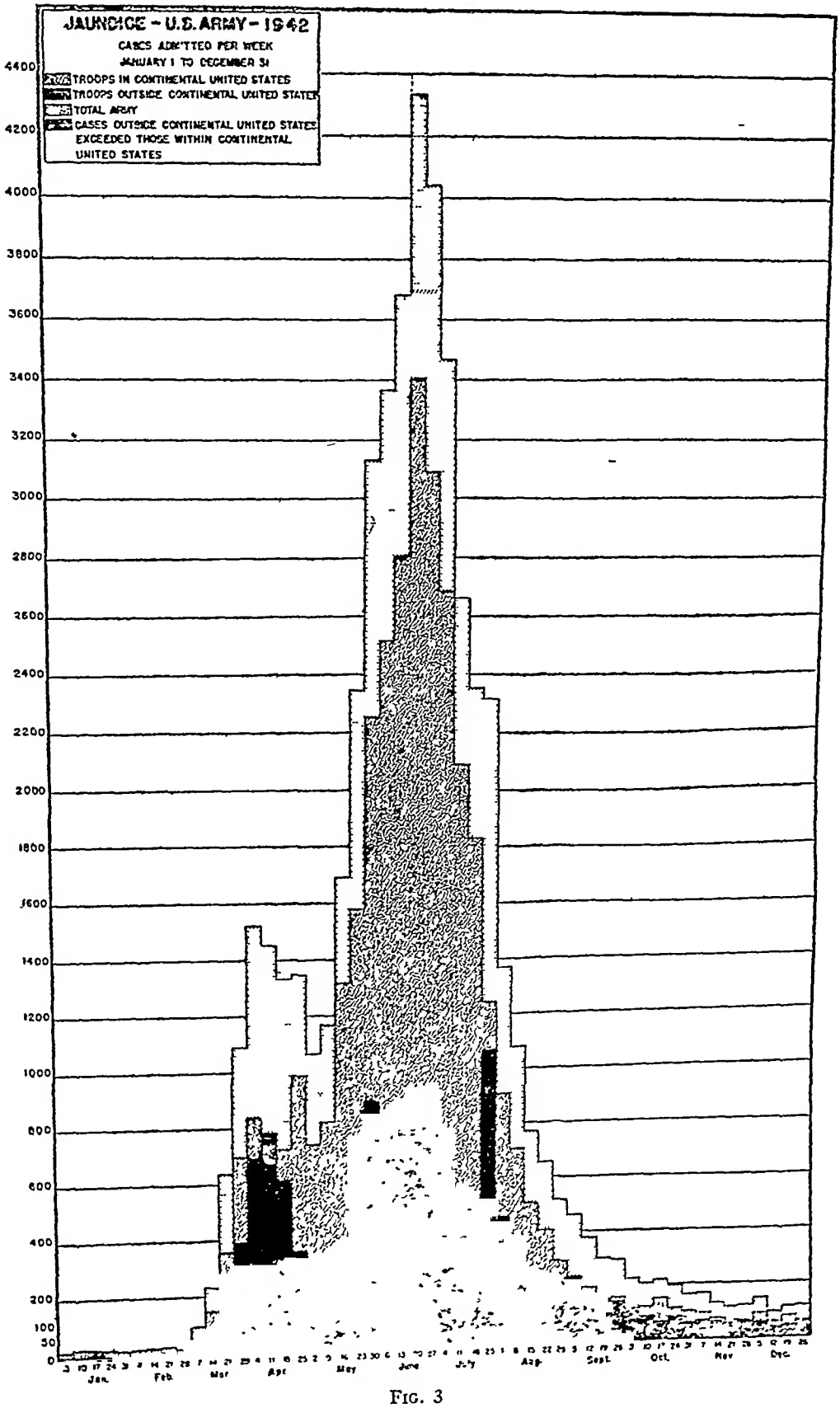
JAUNDICE - U.S. ARMY - 1942
CASES ADMITTED PER WEEK
JANUARY 1 TO DECEMBER 31
TROOPS IN CONTINENTAL UNITED STATES
TROOPS OUTSIDE CONTINENTAL UNITED STATES
TOTAL ARMY
CASES OUTSIDE CONTINENTAL UNITED STATES EXCEEDED THOSE WITHIN CONTINENTAL UNITED STATES
4400
4200
4000
3800
3600
3400
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
100
50
0
Jan.
Feb.
Mar
Apr
May
June
July
Aug
Sept.
Oct.
Nov
Dec.

FIG. 3

368 and 369. It can be seen that a great many non-icterogenic lots of vaccine were administered to persons who later developed jaundice. A ratio of cases of jaundice, per 1000 doses of the vaccine issued, reveals that there is no great variation in the computed attack rate among the non-icterogenic lots. It is believed these cases represent the expected incidence of infectious hepatitis in the Army.

Figure 5 is intensely interesting. It designates the number of doses of yellow fever vaccine by lot numbers that were issued to the Army and Navy. The Navy reported practically no jaundice during 1942. As can be seen from this figure, the Navy received none of the icterogenic lots except 334 and 369. A limited number of doses of these lots were issued to the Navy, and jaundice did occur in personnel immunized with lot 334 of the vaccine.

Figure 6 shows several "work sheet graphs" illustrating the striking resemblance of the vaccination schedules at certain posts to their subsequent outbreaks of jaundice. Even in small isolated posts in Alaska this type of graph obtained.

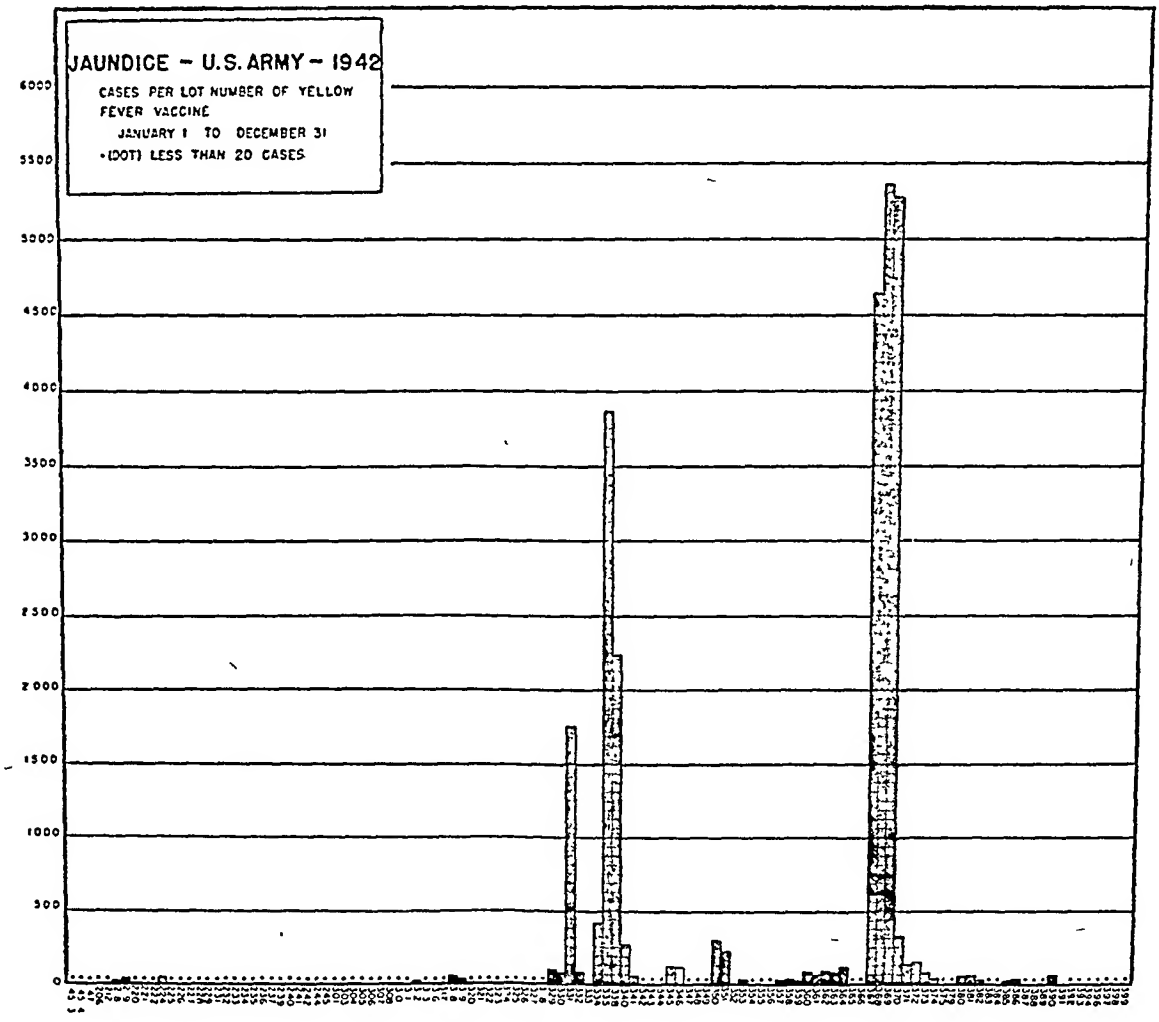

FIG. 4

II. INFECTIOUS HEPATITIS IN THE ARMY IN NORTH AFRICA IN 1943. (NATURALLY OCCURRING INFECTIOUS HEPATITIS)

Let us now turn to the naturally occurring disease. It is generally recognized that infectious hepatitis has a world-wide distribution. It occurs throughout the year, but with a marked seasonal prevalence. A study of the incidence of infectious hepatitis among troops in the United States from 1931 to 1941 reveals that admissions from this

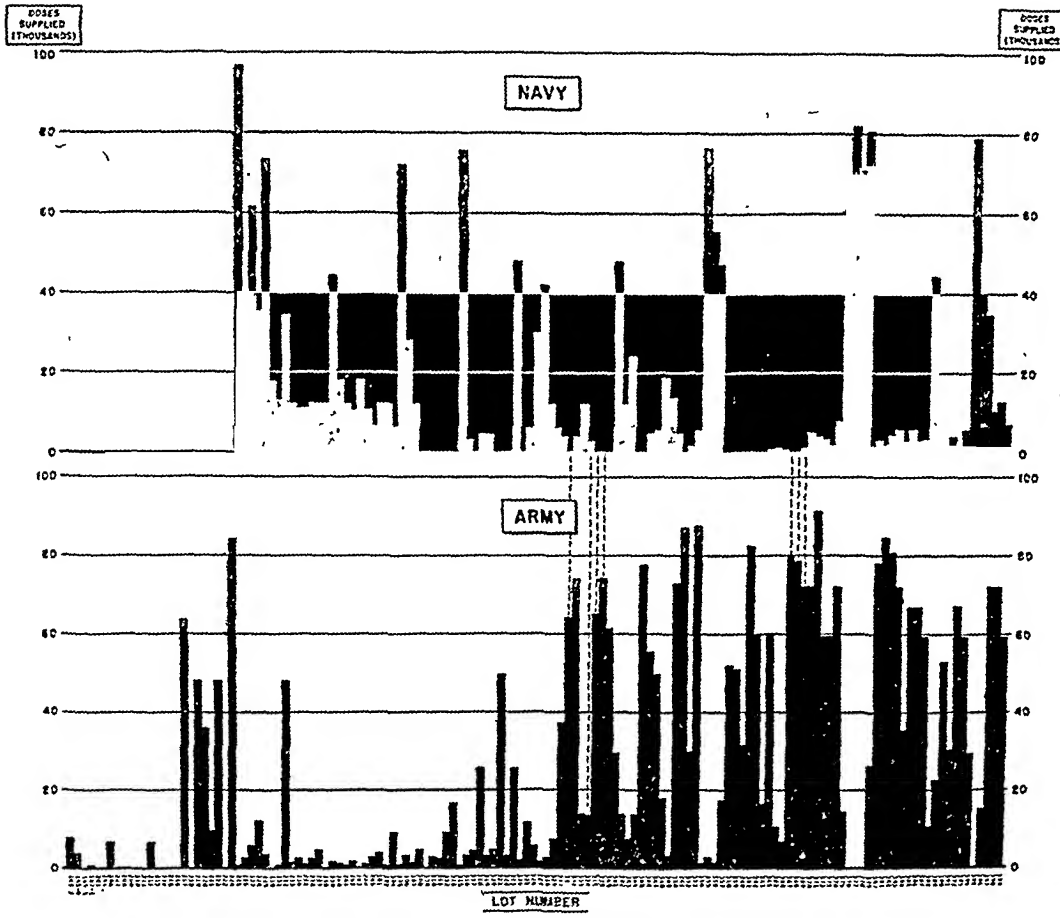

FIG. 5. Doses of yellow fever vaccine supplied to Army and Navy

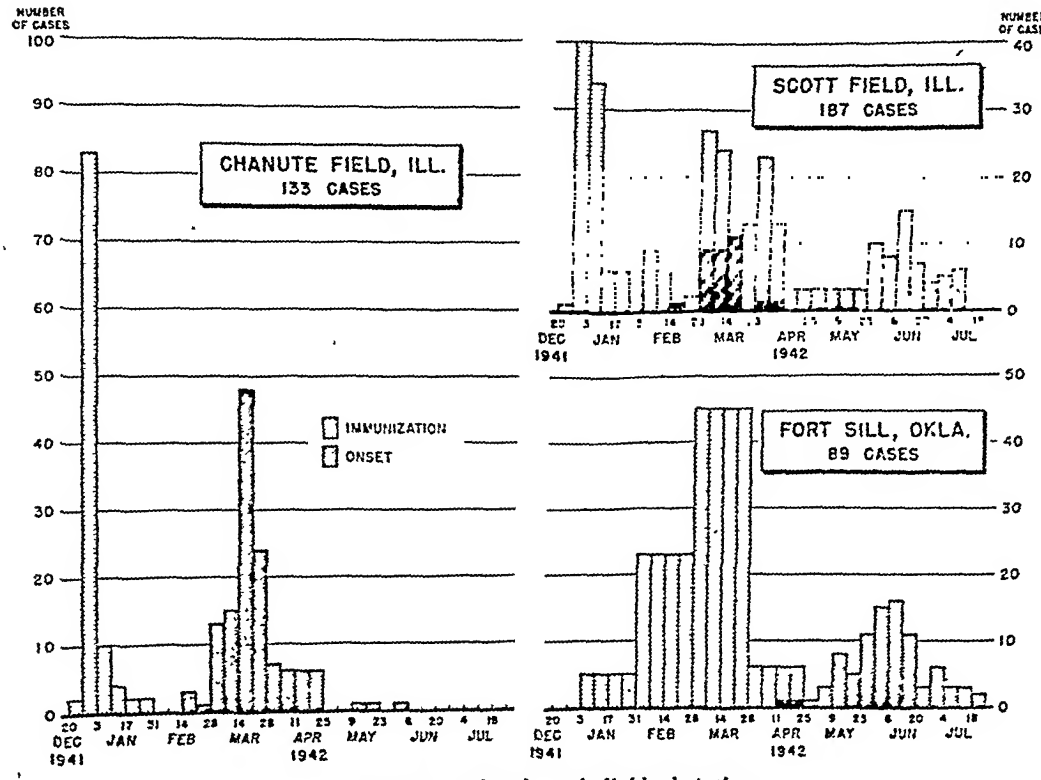

FIG. 6. Jaundice outbreaks at individual stations

disease increase during the late summer, reach a peak in November and December and then fall off sharply to an average level of about 1 per 1000 per annum. It has been noted, however, that, in those regions of the southern hemisphere where seasons are reciprocal to ours, the peak incidence of infectious hepatitis in troops occurs during months corresponding to our autumn.

During the late summer and fall of 1943, a rather extensive epidemic of infectious hepatitis occurred among our troops in North Africa. As can be seen from *Figure 7* admissions from jaundice in this theater, which has seasons corresponding in general with those in this country, began to increase in August and September, reached a peak in November, and then fell off rather sharply, following the usual pattern for this infection. This epidemic provided opportunity for investigation of the naturally occurring disease, and intensive studies were made of its epidemiology by medical officers in the Theater and by specialist personnel from this country. These studies have been continued in laboratories, both in North Africa and in the United States, and certain salient facts have been forthcoming which are forcing a revision of our concepts concerning the origin and spread of infectious hepatitis.

I have mentioned that until recently the spread of infectious hepatitis was believed to be respiratory in nature, and efforts to control it had been directed accordingly. Yet in North Africa admissions from jaundice did not parallel those from respiratory diseases and close contacts with developing cases did not have a higher rate of infection than non-contacts. Proponents of the theory of respiratory spread have pointed out, however, that there may be four times as many non-icteric cases as icteric. Thus the epidemiological picture is complicated because of the impossibility of accurately tracing contacts. Also, experimental evidence to support the theory of respiratory spread is afforded by the work of Finlay and Martin, who produced mild jaundice in human volunteers by instilling into their noses the nasal washings from three patients in the pre-icteric or early stages of hepatitis following yellow fever immunization.

The apparent restriction of the disease in North Africa to certain insect-ridden areas plus the tendency of incidence curves toward a relationship with those of malaria and sandfly fever, but occurring three months later, has led other observers to postulate that infectious hepatitis is transmitted by blood sucking insects, and that the prolonged incubation period observed in post-vaccinal jaundice also obtains in the naturally occurring disease. The theory of insect transmission seems very plausible to them. The facts that jaundice can be transmitted by inoculation of small amounts of serum, that the virus apparently remains in the blood of infected persons for long periods of time, that the disease seems to occur more frequently in certain locations, that there is a definite seasonal incidence and that secondary cases are hard to prove, are all in keeping with this theory. Experimental proof is lacking at the present time, but work now in progress under the direction of the Army Epidemiological Board should provide interesting data in the near future.

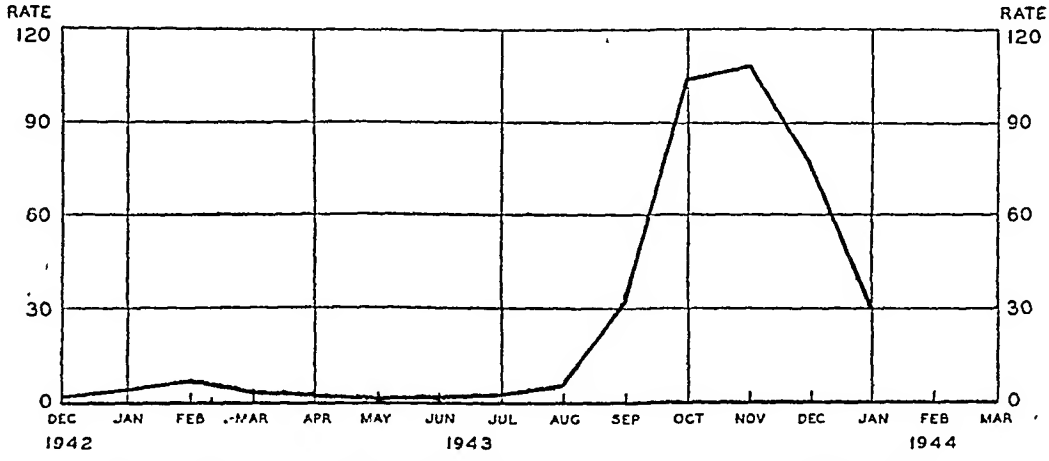

FIG. 7. Infectious Hepatitis, admissions per thousand men per year North Africa

In reviewing conditions in North Africa a month before the jaundice outbreak, still other investigators were led to trace its origin to the same conditions which were associated with high rates of diarrheal disease and dysentery. This observation may be entirely valid, as it now seems likely the spread of infectious hepatitis is related to insanitary conditions. Experiments in both this country and England have recently shown that the virus of infectious hepatitis occurs in human feces. Its spread, therefore, might be expected under the same conditions which favor that of bacillary dysentery, and because of its rather long incubation period would occur a month or more after a dysentery outbreak.

We are therefore in a dilemma peculiarly similar to that presented in the control of poliomyelitis. We are now aware that an attempt to prevent the spread of infectious hepatitis by employing only those methods used to control respiratory infections is probably inadequate. It seems clearly indicated that until the epidemiology of this disease is more completely worked out, we must place more emphasis on environmental sanitation and insect control than has previously been considered necessary.

THE SYNDROME, TROPICAL EOSINOPHILIA AND MICROFILARIA

A. VAN DER SAR AND H. HARTZ

From the Public Health Service, Curaçao, N. W. I.

Received for publication January 8, 1945

In the last 25 years numerous publications have appeared concerning the occurrence of a hyperleukocytosis with hypereosinophilia, concurring clinically with enlargement of superficial lymph nodes a spleen either enlarged or not, and attacks of asthmatic bronchitis. The pathologic picture developed gradually and appeared to have a benign course. Roentgenologically small bronchopneumonic foci could be demonstrated in some cases.

Giffin (1), in 1919, reported a case with persistent eosinophilia, hyperleukocytosis and splenomegalia, in which at the same time enlarged superficial lymph nodes were found. The number of leukocytes was 21,000 with per cu. mm. 73% eosinophilic cells.

In 1921, Aubertin and Giroux (2) reported a case of splenomegalia in which the number of leukocytes varied from 6900–26,000 per cu. mm. with an eosinophilia of 65–70% respectively, and McDonald and Shaw (3) followed, in 1922, the highest number of leukocytes amounting to 31,000, of which 71.4% were eosinophiles. In the Netherlands East-Indies de Langen and Djamil (4), in 1923, observed a patient with a leukocytosis of 13,200 and an eosinophilia of 85%. Here the asthmatic phenomena came to the fore front and a large, hard spleen developed. Antihelminthics had no influence on the course of the disease.

In 1927, Armand-Delille and Mme de Pierredon (5) demonstrated a boy from one of the French colonies, with repeated attacks of asthma. Here, too, the spleen was enlarged, there was persisting leukocytosis of 35,000 cells with an eosinophilia of 76%. Antihelminthics gave no improvement. Little patches were to be seen on the x-ray picture of the lungs which reminded one of miliary tubercles. At the same time a new report of de Langen (6) appeared about *Strongyloides stercoralis* infections with marked leukocytosis. The lowest number of leukocytes was 8000 and the highest 26,000 per cu. mm. The eosinophilia varied from 30–85%, with the asthmatic phenomena predominating. In 11 of 13 patients the spleen was enlarged. The patients were treated with tartar emetic injections and all phenomena disappeared promptly. No relapse was observed.

In Cuba, Valledor, Mendoza and Pendrera (7), in 1939, reported 4 cases in children with hyperleukocytosis, in which the number of leukocytes varied between 20,000 to 71,000 per cu. mm., whereas the highest percentage of eosinophilic cells amounted to 80%. The principal features were: a somewhat enlarged spleen, and slight rises of temperature during several months. The little patients had a dry cough. All the x-ray pictures of the lungs showed the so-called pseudo-granula picture. The children were observed for several years; in all of them the process had a favorable course.

In 1941, Bass (8) observed three cases. Two of them had been published by him in 1931, as presumable eosinophilic leukemias. However, one of them was traced during seven years, the blood-picture recovering itself completely in that time. The boy was a native of Puerto Rico, W. I.

The largest number of leukocytes that was observed amounted to 45,200 per cu. mm., with an eosinophilia of 73%. In this case there were swollen lymph nodes and an enlarged spleen. Lung-phenomena were found in only one patient.

In the Netherlands East-Indies a publication appeared in 1939 by Meyers and Kouwenaar (9) in which a very important discovery was made. In 7 patients with leukocytosis and hypereosinophilia, large glands were found in the groin-region. In one case there existed a hyperleukocytosis of 38,000 per cu. mm., with an eosinophilia of 65%. The lowest number of leukocytes was 7000 per cu. mm., and the lowest percentage of eosinophilics 11%, but in all cases there was general enlargement of the lymph nodes, expecially of the inguinal glands. In three cases abscesses were found in the nodes. In only a few cases was the spleen enlarged. Two patients showed symptoms of acute nephritis, whereas two patients suffered from attacks of bronchial asthma. On accurate and extensive microscopic examination they found in the inguinal glands little eosinophilic abscesses in which there were parasites which had the aspect of microfilaria.

However, no microfilaria was found in the blood (in some cases with an observation period of 2½ to 3½ years). No adult filaria was found. The nephritis and asthma attacks were considered by them as allergic with a filaria-infection as the chief cause. Some weeks later they succeeded in making smears of the glands, in which the microfilaria could be demonstrated as *Microfilaria malayi* (10). There remained the peculiarity that these microfilaria could not be found in the peripheral blood. Weingarten (11), in 1943, in India, mentioned 81 cases of hypereosinophilia which he had observed since 1934. In his table the highest number of leukocytes is 64,000 per cu. mm., the highest number of eosinophilics 89%. He speaks of a new pathologic picture "Tropical Eosinophilia" and emphasizes the lung phenomena, attacks of bronchial asthma, stubborn cough which impedes sleeping and presents itself between 1 a.m. and 5 a.m. During the daytime the patient is free from fits of coughing. Typical symptoms are malaise, lack of appetite and emaciation. During the first period, in which fever occurs, the spleen is enlarged. No mention is made of enlarged lymph nodes.

PERSONAL OBSERVATIONS

Case 1: Patient Z. A., white female, aged 46, born in the Netherlands was treated in February 1942 for a little abscess in the left breast, which healed without any complication. In June, 1942, she was seen for the first time, as she had had a stubborn cough for a month. This cough presented itself in attacks, especially at night between 3 a.m. and 4.30 a.m. These fits of coughing were combined with asthmatoid tightness of the chest. She practically did not cough during day-time. She had light rises of temperature, lack of appetite, was tired, listless, excitable and emaciated. When the patient was on night-duty (she was a nurse) the attacks also occurred but in a lesser degree and on the same hours. She could sleep well during the day-time and by doing so she rested better. During her 18 years stay in Curaçao she had never been seriously ill and had never had any attacks of erysipelas and during these 18 years she had never left the island. There are no asthmatics in the family.

The sputum that was excreted was tough and mucopurulent, never mixed with blood and had no metalic taste. The defecation was normal, and of late she had had no diarrhea. Urination occurred without any complaints, and albumin had never been discovered in the urine, neither had she observed anything resembling chyluria.

Physical examination: The patient who had already been in bed for a week, looked tired and listless. The pulse was regular, equal, symmetrical and felt somewhat weak. The pulse rate was 86 a minute. The systolic blood pressure was 120 mM mercury and the diastolic 90 mM mercury. The skin felt moist, the patient perspired very much, there was no edema and no jaundice. The head showed nothing abnormal. The trachea was situated in the middle, the thyroid-gland was not enlarged, there were no palpable lymph nodes.

The thorax: the lungs expanded evenly on deep inspiration, the lungborders were of equal height and moved well. The lung-liver border was situated in the 5th intercostal space. At percussion the sound was normal. On auscultation both dry and moist rales were heard diffusely over the lungs. The left mamma was a little larger than the right one and a little edematous under the mammilla a scar could be seen.

On percussion the heart was of normal size and not displaced. The heart-sounds were clear; A2 louder than P2. A packet of lymphnodes could be felt in the left axilla, the nodes were not confluent with the skin, neither with the underlying tissues nor with each other. The size varied between that of a pea and that of a large bean. The abdomen was flaccid, the liver was not enlarged and not palpable. The spleen reached as far as the arcus costae, and felt hard.

The abdominal lymphnodes were not palpable. The genitalia externa et interna showed nothing abnormal. The reflexes were normal. The muscles were normally developed, there was no atrophy.

Laboratory examinations: The urine: the specific gravity was 1022, the urine was clear and of a yellow colour. The reactions on albumen, glucose were negative, the urobiline reaction slightly positive. The sediment contained 0-2 leukocytes per high power field. The sputum was repeatedly examined for tbc bacilli; always with negative result.

The sputum contained a remarkable number of eosinophilic cells. The examination for fungi both in the microscopic preparation as in the culture test was negative.

The sputum examination for parasitic ova, for larvae of anguillula stercoralis and for microfilaria remained repeatedly without positive results. The examination for microfilaria in the capillary blood taken every two hours during 24 hours, was negative, just as the concentration method with venous blood on various dates during the period from June 1942 to July 1944.

The feces examination for ova and parasites was negative. The concentration methods of Cort Sand-Ground for anguillula stercoralis gave always negative results.

The reactions for trichina infection with the skin test as well as the complement fixation test were negative as was Casoni's reaction for echinococcus. The tuberculin reaction (Pirquet) was positive. The chemical examination of the serum gave the following results: the total cholesterol content 229.8 mgr.% as esters 152 mgr.%. The bilirubin content according to Hymans

van den Bergh: direct reaction negative, indirect ½ Unit.

The Takata-Ara reaction was positive. The total serum-protein determination gave 8.1% of which albumin 50.8% and globulin 49%. The Wassermann and Kahn reactions were negative. The determination of the metabolic rate gave the value + 4%.

Biopsy of the lymph nodes in the left axilla was performed by Dr. M. J. Hugenholtz.

Examination of the biopsy: Macroscopic. On section the nodes were pale brownish grey in color and showed several minute yellowish spots which were softer than the surrounding tissue. The nodes were embedded in fairly dense, greyish connective tissue. Microscopical examination: The original structure of the node could only partially be recognised; the marginal sinus had disappeared in many places and the lymphoid tissue bordered directly on connective tissue rich in collagen and heavily infiltrated by plasma cells and in some places by eosinophilic leucocytes.

In the fatty tissue found in the neighbourhood of the nodes the infiltration was particularly heavy. Here enormous numbers of plasma cells and smaller, but still very great numbers, of eosinophilic leukocytes were present; here also new follicles with germ centers had been formed. In the nodes there were great numbers of follicles with germ centers which often consisted almost completely of large reticulum cells and histiocytes and generally showed few mitoses.

The lymphatic tissue contained many plasma cells. The majority of the intermediate sinuses were narrow and their reticulum could be easily discerned.

Eosinophilic polymorphonuclear leukocytes with the typical bilobed nucleus were found in great, sometimes enormous numbers, in the nodes. One or more of them were found lying in nearly every blood vessel; they were present in varying numbers in the sinuses but the greatest number occurred in the lymphatic tissue. Here they were found as loose eosinophilic infiltrates, which in some places were more dense and circumscript and in still other places formed eosinophilic abscesses. Here many eosinophilic leukocytes showed signs of disintegration. Eosinophilic myelocytes were not observed.

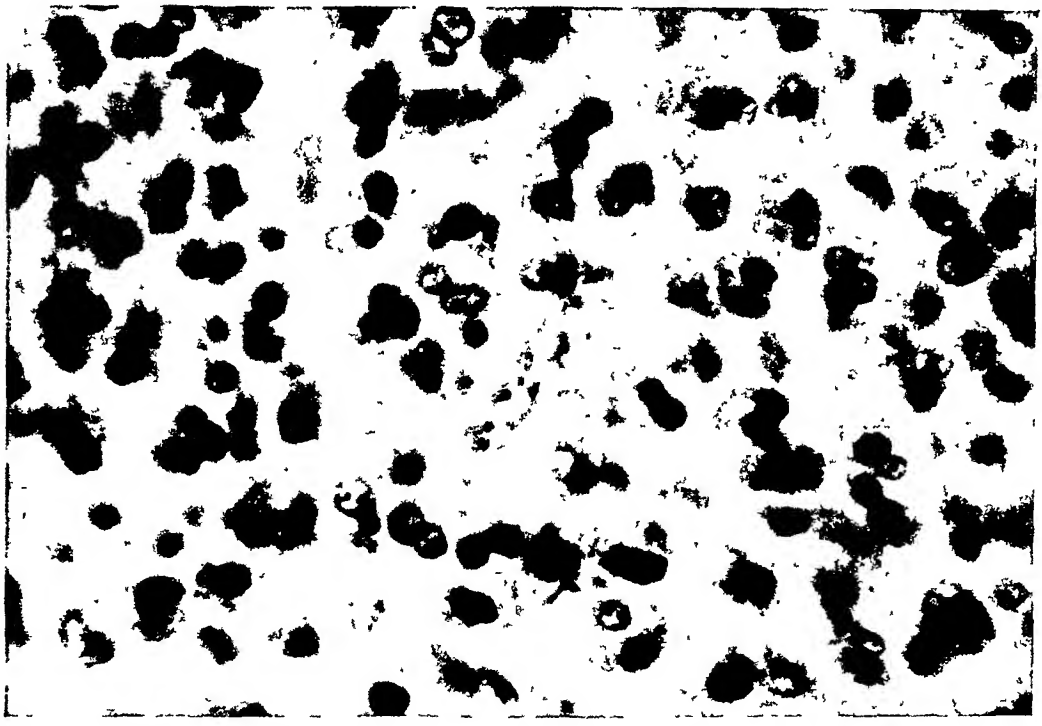

FIG. 1. Case 1. Part of microfilaria probably tail, surrounded by eosinophilic leukocytes. ×1300.

In some of the eosinophilic abscesses and infiltrates small worms were observed which showed the characteristics of microfilaria They differed only from the microfilaria which are found in the lumina of blood vessels in that they appeared to be more shrunken and probably must be considered as dead.

Course: As the patient was continually being troubled by asthmogenic bronchitis, it was decided in Feb. 1943 to treat her with tartar emetic injections. The tartar emetic was dissolved in 5% glucose solution. The total quantity was 1500 mgr. After 4 injections the effect was brilliant, the attacks decreased in violence and frequency, she excreted less sputum and could sleep better. She had not had any more attacks after the 6th

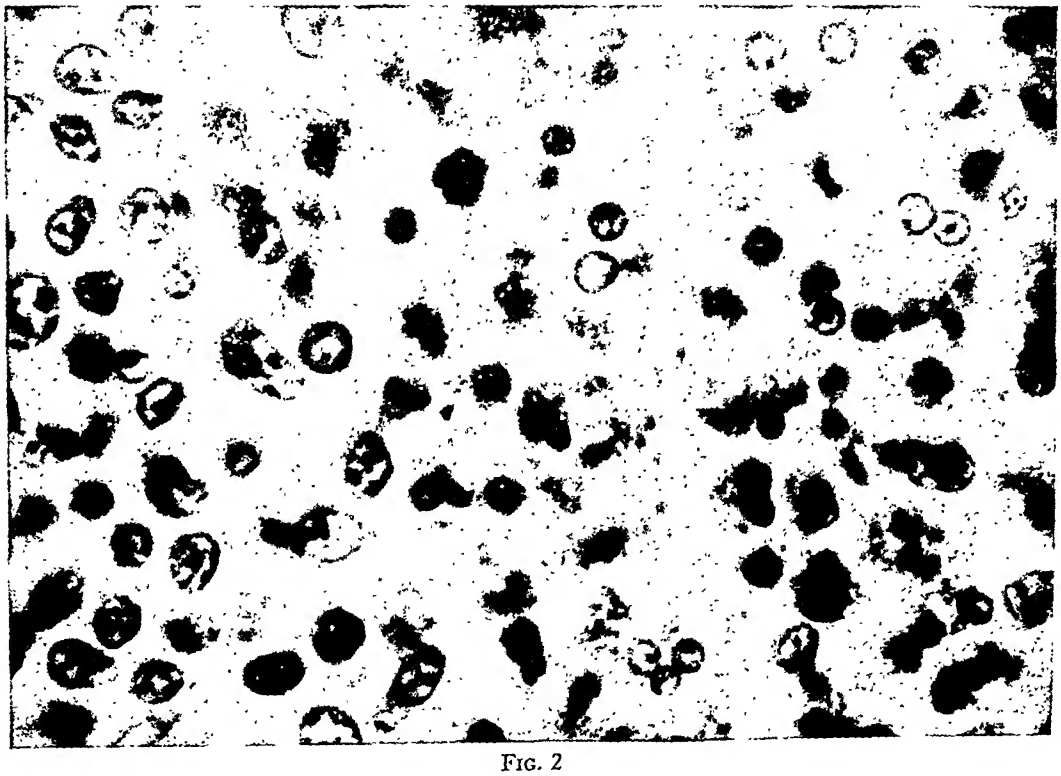

FIG. 2

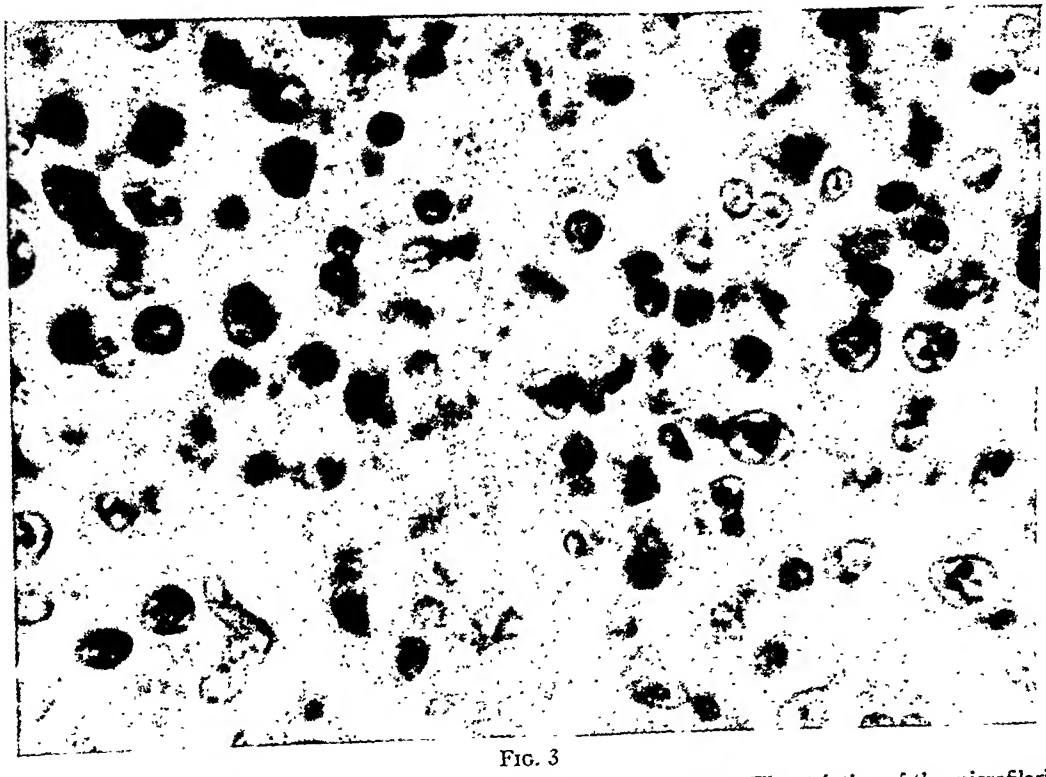

FIG. 3

FIGS. 2 and 3. Case 1. Section of microfilaria focussed in different planes. The striation of the microfilaria or s cuticula is visible. There are many eosinophilic leukocytes. ×1300.

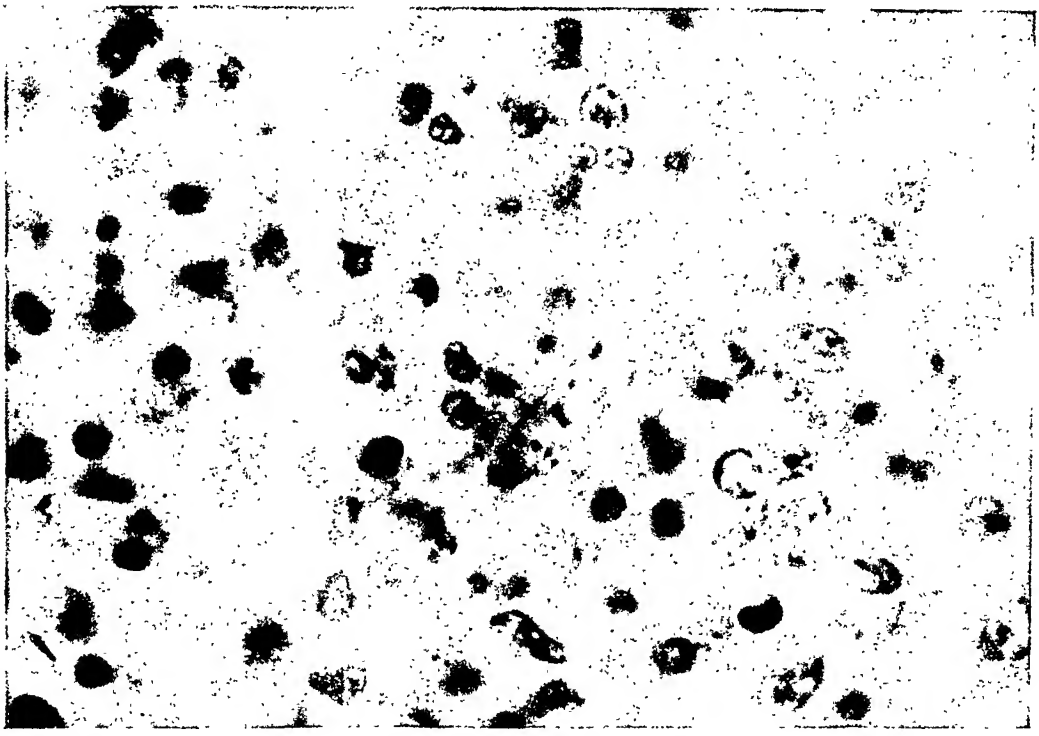

FIG. 4. CASE 1. PART OF MICROFILARIA. ×1300

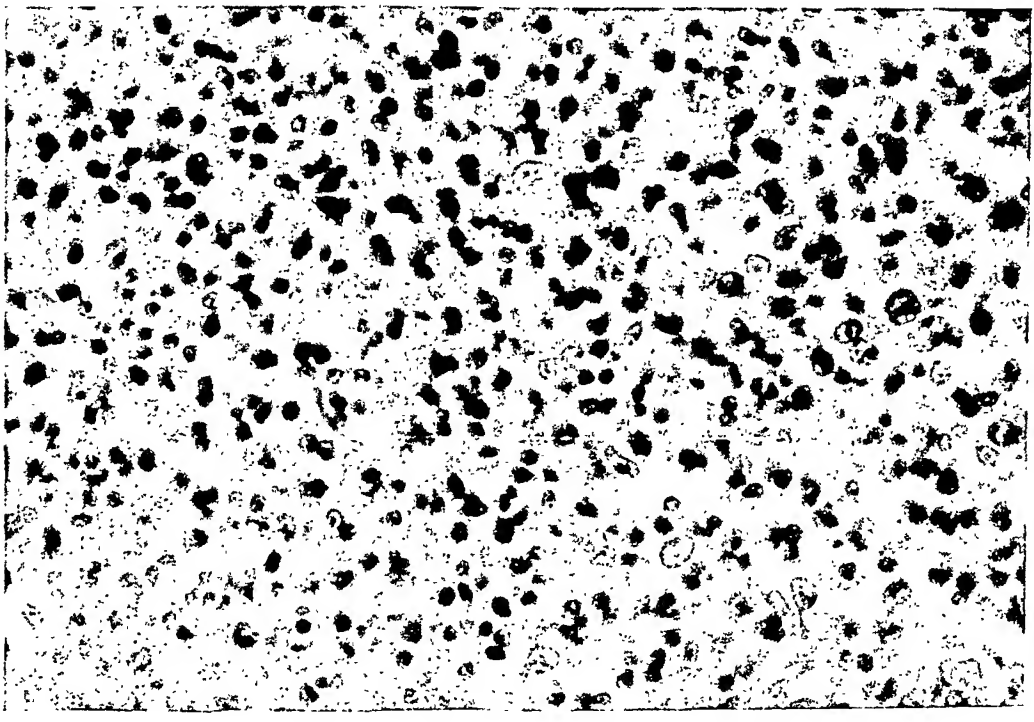

FIG. 5. CASE 1. EOSINOPHILIC INFILTRATE. ×1300

injection. The patient started to eat better, felt better, grew more cheerful.

Light toxic phenomena were observed only at the end of the cure in the form of muscular weakness. High doses of vitamin B1 caused the phenomena to soon disappear. The effect on the blood-picture is clearly visible in the table (Table 1). The number of leukocytes has decreased from 13,600 to 9,500 per cu. mm., the number of eosinophilic cells from 49% to 11%; the rate of sedimentation according to Westergren, from 51 mm. the first hour to 24 mm. the first hour. The total serum-protein was now 5.2% of which albumin 51.%, globulin 47%, the fibrinogen 3.3%. The Takata-Ara reaction was still positive. Cholesterol total 170 mgr.%, cholesterol ester 100 mgr.%. The patient was completely free from complaints until the end of May, 1943, when on the 25th of May, 1943, she had once more an attack (for the blood picture see Table 1). On August 15th, 1943, the Mafarside cure according to Weingarten was begun. The bronchial phenomena disappeared immediately. From August, 1943, until Dec., 1944, the patient has had no more attacks. Her weight has considerably increased from 55 kg. to 68 kg. The blood picture has completely changed, although the number of eosinophilic cells is still a little too high. The Takata-Ara reaction is at present negative. No microfilaria could be shown in the peripheral blood.

Case 2. Patient O. S., a white man, aged 31, born in Curaçao, was seen by us for the first time when he had to be examined for Government service. In the anamnesis he indicated that from Dec., 1942, he had suffered from asthma attacks. He coughed much, also during the daytime. He expectorated muco-purulent sputum but had never seen blood with it. The sputum had no metalic taste. The time of appearance of the attacks depended upon the time at which he went to bed; did he go early, then the attack came at 2 a.m.; did he go to bed late, i.e. 12 p.m., then the attack came at 6 a.m. For that reason he generally went to bed late in order to get a sufficient night's rest.

Since Jan., 1943, he had noticed that there were lymph nodes in the right axilla. He had never had any fever. Defecation was regular and he had never observed any diarrhea. Micturition was normal, nephritis or chyluria had not been found. He had never had venereal diseases. At the age of 6 he had gone to Venezuela for a period of 4 years, after which attacks of asthma had manifested themselves until his 15th year. When he was 17 years old he developed pleuritis

TABLE 1

Results of Blood Counts in Case 1

DATE	HEMOGLOBIN CONTENT	RED CELLS, PER CU. MM.	WHITE CELLS, PER CU. MM.	POLYMORPHONUCLEAR NEUTROPHILS	NONSEGMENTED NEUTROPHILS	EOSINOPHILS	BASOPHILS	LYMPHOCYTES	MONONUCLEAR LEUKOCYTES	SEDIMENTATION RATE
	per cent			*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*mM*
6/ 3/1942	76	4,620,000	16100	29	0.1	59	0.3	0.8		42
6/11/1942	73	3,220,000	23300	16	0.1	62	0.1	18	2	50
6/19/1942	77	4,600,000	25100	23	0.1	62		13	1	
7/ 3/1942	75	3,990,000	20900	36	0.2	34	0.3	19	6	91
7/14/1942	78	4,900,000	28100	27	0.1	52		15	5	40
8/ 7/1942	78	4,940,000	23100	22	0.1	49	0.1	20	7	78
9/ 5/1942	63	3,860,000	18400	41	0.1	39	0.1	13	5	74
1/ 7/1943	82	4,280,000	26300	25		57	0.1	11	6	60
Tartar emetic course										
2/ 2/1943	75	4,180,000	13600	37		49		11	3	51
2/13/1943			10700	29		43		25	3	30
3/ 1/1943	69	4,190,000	7600	32	0.1	40	0.2	22	3	24
3/15/1943	81	4,020,000	9500	52		11	0.2	30	5	
Tartar emetic course finished										
5/27/1943	80	4,500,000	21500	31		61		0.7	1	51
8/14/1943	Mafarside course									
8/14/1943	91	4,880,000	18800	35		53	0.1	0.9	2	0.5
9/23/1943			10900	37		19	0.1	37	6	
11/ 3/1943	90	4,610,000	11600	41		16		41	2	10
Mafarside course finished										
2/24/1944	84	4,570,000	8400	48		0.9		36	7	
7/26/1944	97	4,850,000	9900	50		0.7		33	10	0.5

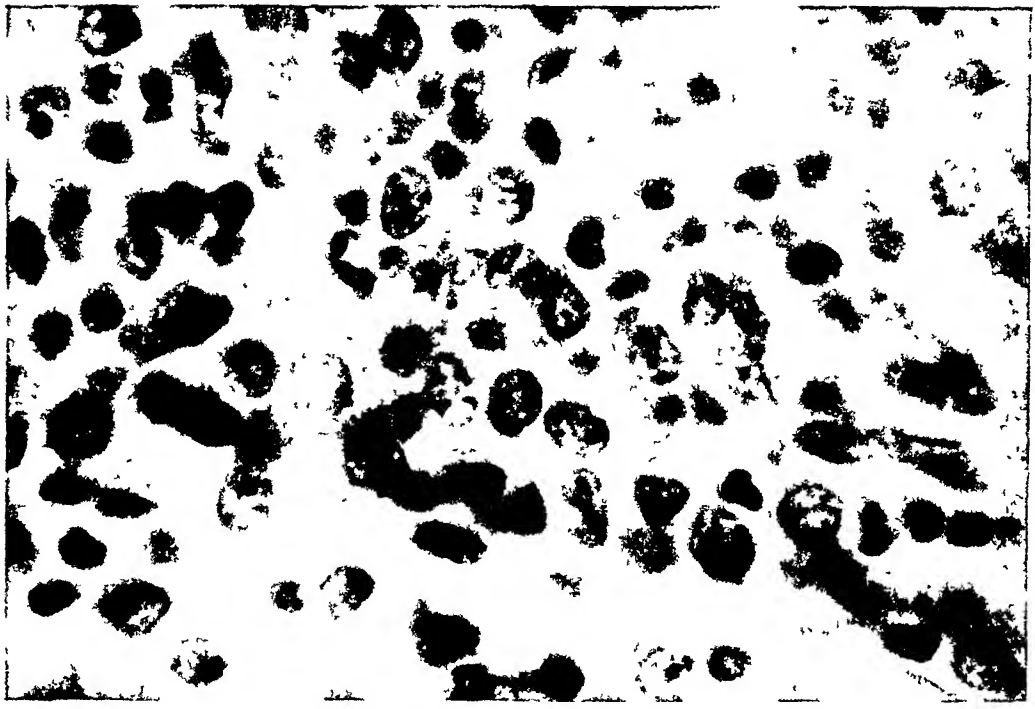

FIG. 6. Case 2. Eosinophilic myelocytes in a lymphnode. ×1300.

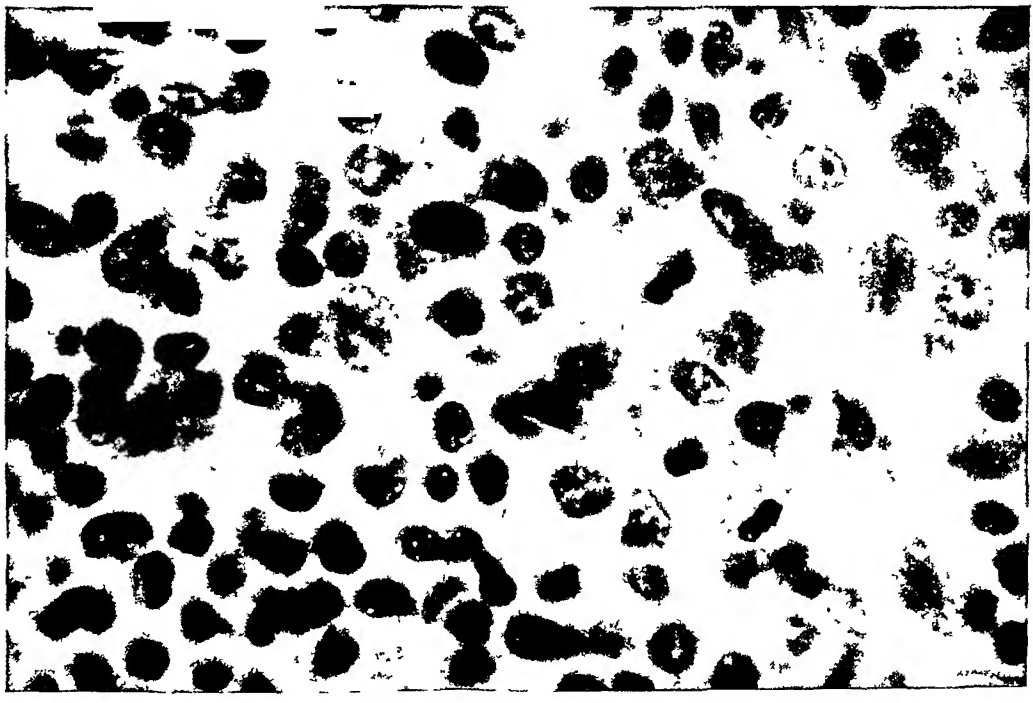

FIG. 7. CASE 2. MITOSIS IN EOSINOPHILIC MYELOCYTES. ×1300.

sicca and this disease lasted 6 weeks before he was completely recovered. From his 15th up to his 31st year he had never had any more trouble from asthma attacks. In the family anamnesis no asthma or other allergic diseases occurred.

Physical examination: The patient was a well-built,

muscular man. Nothing abnormal could be found on the head and neck. The tonsils were not enlarged. The pulse was regular, equal, symmetrical, the pulse rate was 80 a minute. The blood pressure was 120 mm. systolic and 80 diastolic. The thorax was wide and moved evenly. The lung borders were equal in height and moved well. The percussion sound was normal. On auscultation there were dry and humid rales with an expiratory wheeze. The heart configuration was normal. On auscultation no murmurs were heard.

The examination of the abdomen showed a palpable spleen, just one finger beneath the arcus costae. The reflexes were normal. The genitalia externa were normal.

fixation test for trichinosis were negative. The total serum-protein content was 9.5% of which albumin 37.2% and globulin 62.8%. The Takata-Ara reaction was positive. The total cholesterol content was 170 mgr.%, cholesterol ester 100 mgr.%. The liver function test (galactose test) was negative. The basal metabolic rate was plus 17%.

The bilirubin content of the blood serum according to Hymans van den B Bergh, was not increased. The serological reactions for lues were negative. The first blood picture gave 41,000 leucocytes per cu. mm., with 55% eosinophilic cells (see Table 2).

The sputum examination for tubercle bacilli was negative, but many eosinophilic cells were present.

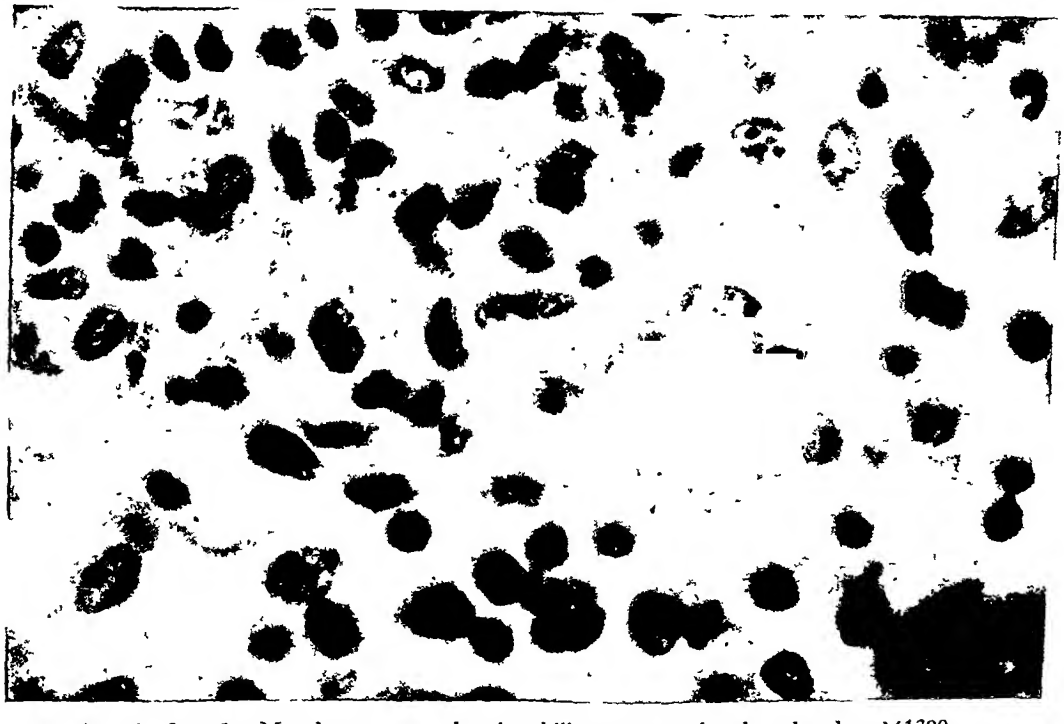

FIG. 8. Case 2. Megakaryocyte and eosinophilic myelocyte in a lymphnode. ×1300.

Lymph gland system: In both axillas enlarged lymph glands were palpable. In the right axilla one gland of the size of a dove's egg was palpable and it was hard and adherent to the large vessels. In the left axilla three was one gland of the size of a large bean and some smaller ones; they were not fused and were very easily movable. The x-ray picture of the lung showed large hili, many bronchial shadows and diffusely scattered small foci.

Laboratory and Chemical examinations. The urine examination. specific gravity 1025, the reactions for albumin, glucose and urobiline were negative; the sediment contained nothing abnormal. The feces examination for parasite-ova, repeated several times, gave negative results. The skin reactions for tuberculosis, trichinosis and echinococcus, as well the complement-

No *Strongyloides* larvae or micro-filaria were found in the sputum.

Biopsy of the lymph nodes in the left axilla was performed by Dr. M. J. Hugenholtz.

Microscopical examination: The structure of the node was typical. The capsule was thin and in some places infiltrated by plasma cells; there was a narrow marginal sinus. In the cortex there were well developed follicles with pale centers; sometimes these contained much hyaline material. The intermediate sinus appeared normal.

Eosinophilic polymorphnuclear leukocytes were present in very great numbers; they were found in the lumina of the blood vessels, were very frequent in the sinus and occurred in the lymphoid tissue as eosinophilic infiltrates and as small eosinophilic abscesses.

In another part of the node the histological picture was entirely different. Though the general structure of the node could still be easily recognized, there were no follicles. Chiefly in the medullary cords but sometimes to a much lesser extent in the sinus, there occurred large numbers of eosinophilic myelocytes with pale staining, round to oval nuclei with one or two nucleoli. They not infrequently showed mitotic divisions. There were also typical eosinophilic meta-myelocytes and leukocytes. In several places large cells with very fine, already eosinophilic granules, were seen which were probably pro-myelocytes. There were several well developed megakaryocytes. The lymphoid tissue appeared to be overgrown by the myelocytes. As unfortunately the available material had already been serially sectioned and stained with hematoxylin and azophloxin, hematologic methods could not be employed and no attempt was made to trace the promyelocytes, myelocytes and megakaryocytes to a well defined stem cell. No erythropoiesis and no cells which could be identified as neutrophilic myelocytes were observed.

In the material from this patient no microfilaria could be found.

Course: Violent asthma attacks manifested themselves after the extirpation of the glands and these were treated symptomatically. In March, 1943, the tartar emetic cure was started. After the fourth injection no more asthma attacks were observed. Phenomena of intoxication were not noted. At the end of the cure (total quantity of tartar emetic 1500 mgr.) the number of leukocytes had decreased to 12,400 per cu. mm., and the number of eosinophilic cells to 17%. The sedimentation rate (Westergren method) changed from 36 mm. the first hour to 25 mm. the first hour. The total serum-protein content was now 8.7% of which albumin 52.2% and globulin 47.5%. The Takata-Ara reaction was positive. From April, 1943, until Dec., 1944, the blood was counted once in a while and the leukocyte values have decreased to normal but the number of eosinophilic cells is a little too high. The sedimentation rate has become normal. He has not had any more asthmatic attacks. His weight has increased from 161 lbs. to 185 lbs. The lymph nodes have totally disappeared. The man feels fit and plays again his tennis matches.

Case 3: A colored boy, 17 years of age, was admitted to St. Elizabeth's Hospital in July, 1938, on account of gland-tumors in the sulcus bicipitalis medialis dextra (just above the elbow-articulation) and enlarged glands under the chin. During the last month the glands had been enlarging all the time. He had not had any fever. He coughed but he had not expectorated blood. There had been no asthmatic attacks. He had always been a healthy boy and had never been seriously ill. The defecation was regular and he had never observed any diarrhea. The micturition was normal.

TABLE 2

Results of Blood Counts in Case 2

DATE	HEMOGLOBIN CONTENT	RED CELLS, PER CU. MM.	WHITE CELLS, PER CU. MM.	PLATELETS, PER CU. MM.	POLYMORPHONUCLEAR NEUTROPHILS	EOSINOPHILS	BASOPHILS	LYMPHOCYTES	MONONUCLEAR LEUKOCYTES	SEDIMENTATION RATE
	per cent				*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*mM*
2/ 5/1943	97	5,520,000	41,000	254.000	19	55		24	2	36
2/20/1943			30,200		25	50	1	21	3	
3/12/1943			32,000		6	81		13		
3/22/1943	Tartar emetic course									
3/29/1943	83	4,320,000	18,700	350.000	28	54		16	2	28
4/ 9/1943	80	4,440,000	12,400		52	17	1	22	8	25
	Tartar emetic course finished									
9/27/1943	100	5,270,000	12,600		48	16		49	2	5
12/ 3/1943	99	5,420,000	10,200	320.000	42	10		44	4	6
7/19/1944	97	4,940,000	6,400		51	13	1	30	5	9

Physical examination: The boy is a strongly built lad. On examining the head a lymph node of the size of a large bean was palpable under the chin, and was easily movable on the deeper layers. The examination of the heart and lungs showed nothing particular. On examining the abdomen the liver and spleen were not palpable. In the sulcus bicipitalis medialis of the right arm two big lymph nodes were palpable, one of which was of the size of a dove's egg. The glands were hard, movable on the deeper layers and not fused. The reflexes were normal. The lung picture showed, too, enlarged shadows in the right hilum.

Laboratory examinations: The urine contained nothing abnormal. All examinations of the stools for ova and parasites were negative. The Pirquet test gave a negative result. The Wassermann and Kahn reaction for lues were negative. The examination of the periph-

eral blood for filaria was negative. The blood pictures are united in Table 3.

Biopsy of one of the lymph nodes in the sulcus bicipitalis medialis was performed by Dr. M. J. Hugenholtz. A well-known foreign pathologist had made a diagnosis of Hodgkin's disease. Microscopical examination of the first biopsy (1938). Of this biopsy only 2 sections were available. The biopsy showed lymphoid tissue consisting of large follicles with germ centers embedded in partly hyalinized connective tissue. The rest of the regular stricture of the lymph node could only be recognized in a few places.

In the germ centers peculiar giant cells were found, consisting of a conglomerate of 4–8 fairly large, pale staining nuclei with relatively little, basophilic cytoplasm. The lymphoid tissue and the connective tissue were infiltrated by enormous numbers of eosinophilic leukocytes; there were also several eosinophilic abscesses. At the edge of one of the eosinophilic infiltrates a body of the size and the shape of a microfilaria, but without the typical nuclei, was found; it was partly surrounded by a very narrow rim of a homogenous, apparently necrotic, substance. Its nature could not be ascertained.

Second biopsy (1941). This biopsy showed a lymph node, the medulla of which had been almost completely replaced by connective tissue. The lymphoid tissue showed large follicles, sometimes containing well developed germ centers and partially surrounded by lymphatic sinus with a reticulum. The germ centers contained reticulum cells, histiocytes, lymphocytes and many giant cells of the type described in biopsy No. 1. However, in the second biopsy the giant cells were often larger; they often contained up to 20 closely packed, fairly large, pale nuclei and only little cytoplasm. Their form was often elongated and band-like. In the connective tissue isolated strips of sinus, still containing their typical reticulum, were found. Only in a few places there were small accumulations of eosinophilic leukocytes. Microfilaria could not be found.

Course: The patient was treated with Liquor Fowleri. His general condition remained stationary. In January, 1941, the patient was once more admitted, as, considering the favorable course (the lymph nodes had become a little smaller) the diagnosis Hodgkin's disease became doubtful. The remaining gland in the sulcus bicipitalis was removed. An aspiration biopsy of the sternum gave an active bone marrow with a preponderance of the eosinophils. Skin tests for trichinosis and echinococcus were negative as was the complement fixation test for trichinosis. At the second microscopic examination it became clear that the diagnosis of Hodgkin's disease could be abandoned and that here too, considering the great histological resemblances, we had to deal with a case of microfilaria, although we have not succeeded in proving this microscopically. The boy still enjoys perfect health.

Case 4: In this case, in which an automobile accident was the cause of death, strong eosinophilia with microfilaria were found on the microscopical examination of the enlarged spleen. In the liver, too, and in the gastric wall there was eosinophilia so that we may accept that a blood eosinophilia had also existed. From the parents we learned that the boy (7 years of age and born in Curaçao) had been troubled by bronchitis the last few months, any time he had a cold, and when this was the case he had asthmatic attacks during the night. In May, 1944, they had consulted a doctor for the first time as he did not want to eat any more and once in a while he complained of pain in the abdomen. On examination on the 26th of May, 1944, an anemia was found to exist (Hb. 54%). Examination of the feces for amebae, amebic cysts, and helminthic ova was negative. Further investigation was not possible. An examination of the members of the family gave a leukocytosis with strong eosinophilia (see Table 4), which cannot be accounted for by the negative result of the feces examination on ova and parasites. The blood examination for filaria was negative. It is quite possible that these members of the family are also infected by filaria, whereas the leukocytosis with eosinophilia and the anemia also indicate that the same pathological picture is going to develop here. The serological reac-

TABLE 3

Results of Blood Counts in Case 3

DATE	HEMOGLOBIN CONTENT	RED CELLS, PER CU. MM.	WHITE CELLS, PER CU. MM.	POLYMORPHONUCLEAR NEUTROPHILS	NONSEGMENTED NEUTROPHILS	EOSINOPHILS	BASOPHILS	LYMPHOCYTES	MONONUCLEAR LEUKOCYTES	SEDIMENTATION RATE
	per cent			*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*mm*
7/ 9/1938	80	4,170,000	7000	23		53		20	4	
6/ 6/1939			2800	11	0.2	39		44	4	25
11/27/1939			3300	19		38	0.1	37	5	
10/ 7/1941	80	4,830,000	3900	27	0.1	21		44	7	
7/ 9/1943			2900	34		0.9		49	8	9

tions for lues in the mother were negative; also in the deceased boy no signs of lues were found at the autopsy.

We are indebted to the staff of the Medical Department of the Shell Refinery for this information.

Case 4: Autopsy. At autopsy the spleen was enlarged, weighing 60 gram (normal weight for a Curaçao boy of this age plus minus 40 gram). The capsule of the spleen showed many greyish-red, sometimes slightly elevated, spots and numerous hardly visible, villous excrescences. On the cut surface the capsule appeared to be thickened, the greyish-red tissue extending here and there a few millimeters into the pulp; it could be easily distinguished from the thin trabeculae. The liver weighed 730 grams; the gastric mucosa showed many distinct follicles.

TABLE 4

Results of Blood Counts in the Members of the Family of Case 4

	DATE	HEMOGLOBIN CONTENT	RED CELLS, PER CU. MM.	WHITE CELLS, PER CU. MM.	POLYMORPHONUCLEAR NEUTROPHILS	NONSEGMENTED NEUTROPHILS	EOSINOPHILS	BASOPHILS	LYMPHOCYTES	MONONUCLEAR LEUKOCYTES	SEDIMENTATION RATE
		per cent			*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*mM*
M. L.	7/31/1944	80	4,900,000	17700	55	0.3	0.4		30	8	
E. L.	7/31/1944	67	4,930,000	13500	51	0.4	15		26	4	
	8/14/1944	58		11900	51	0.1	11	0.1	34	2	12
S. L.	7/29/1944	58	4,870,000	17000	55	0.3	12	0.1	22	7	
	8/14/1944	64		18200	42		10		47	1	0.2

Microscopical examination: 1. *Spleen:* The capsule was thickened and in some places infiltrated by a few eosinophilic leukocytes and by lymphocytes. The minute excrescences consisted of loose connective tissue, covered by mesothelium, and sometimes infiltrated by eosinophilic leukocytes. The subcapsular pulp showed an increase of the connective tissue, which formed loosely arranged, broad bundles which anastomosed with each other.

Only a few sinuses could be recognized. Eosinophilic leukocytes occurred here in very great numbers. They were either scattered throughout the subcapsular pulp or were lying together as dense, more or less rounded infiltrates. Real eosinophilic abscesses were not seen. In the more dense infiltrates the esosinophilic leukocytes were often associated with cells resembling epitheloid cells and with small atypical giant cells, which consisted of a conglomerate of nuclei with very little cytoplasm. In several of these infiltrates microfilaria were found.

The rest of the splenic tissue showed nothing abnormal. However, the number of eosinophilic leukocytes found here, though not so high as in the subcapsular pulp, was markedly elevated.

2. *Liver:* The sinusoids of the liver contained little blood and few leukocytes. The majority of them were eosinophilic leukocytes.

3. *Mesenteric lymph node.* Isolated eosinophilic leukocytes occurred throughout the node. In one place there was a small nodule composed of swollen reticulum cells and eosinophilic leukocytes. No microfilaria were found.

4. *Gastric Mucosa:* There were many follicles scattered throughout the mucous membrane but especially at the bases of the glands there were many eosinophilic leukocytes.

COMMENT

The microscopical examination of the material from Case 4 shows an almost complete conformity with the case of Bonne (12). In Case 4 the spleen was enlarged and the filaria were only found in the subcapsular pulp, whereas in Bonne's case the spleen was too small and the changes occurred throughout the whole organ. In our case the hypereosinophilia was also found in other organs and the blood in the sinusoids of the liver contained an increased number of eosinophilic leukocytes; it is therefore probable that hypereosinophilia of the blood existed just as in the other members of the family of the patient. The spleen in this case also shows a certain resemblance to the cases of Dhayagude and Amin (13); in their cases the lesions of the spleen were localized and slightly elevated spots were noted in the capsule. However, the lesions were larger and the eosinophilia much less pronounced. In our Case 4 the same atypical giant cells were found as in the cases of Bonne and of Dhayagude and Amin.

As regards Case 3, only a few sections had been examined in 1938. We could not reach a diagnosis and at that time there were no facilities for the production of serial sections. On the authority of a well known foreign pathologist, who visited our laboratory, Hodgkin's disease was assumed, which diagnosis had to be rejected later on, as the clinical

course was not consistent with it. At the time of the second biopsy there was too much fibrosis to expect much success in the search for microfilaria which were not found, though a great number of blocks and sections was examined. The fibrosis, which was already present at the time of the first biopsy, later dominated the histological picture.

The atypical giant cells found in this case deserve special attention. In the cases of Bonne and of Dhayagude and Amin and in our Case 4, they were present associated with the microfilaria. However, in Case 3, they were found in great numbers in the abscence of microfilaria, or, if the microfilaria had been present in the first biopsy, not in close contact with the worms. Perhaps microfilaria would have been found in this case if serial sections had been made of material from the first biopsy.

Case 2 is especially important from the morphological point of view. Local myeloid metaplasia in peripheral lymph nodes, as the axillary and supraclavicular lymph nodes, has only rarely been found. Lang (14) (in Downey's Handbook) mentions the cases of Aschoff (myeloid metaplasia of the axillary lymph nodes of a healthy man) and Roller (myeloid transformation of the supraclavicular nodes within the obstruction area of thrombosed subclavian and innominate veins). Our Case 2 proves that in cases of high eosinophilia the eosinophilic leukocytes need not originate exclusively from the bone marrow but can also be formed in other places, e.g. peripheral lymph glands, though the process of hemopoiesis is typical and homoplastic and no eosinophilic leukocytes are formed directly by the transformation of connective tissue cells, as is sometimes erronously assumed.

Case 1 is completely identical with the cases of Meyers and Kouwenaar. Macroscopically the minute yellow abscesses were visible in the nodes. In the eosinophilic abscesses and infiltrates microfilaria were found. We believe that apart from the changes in the preexisting nodes, caused by infiltration and fibrosis, new lymphoid tissue had also been formed.

The ages of the 4 patients were 7, 17, 33 and 48 years, respectively, and age does not seem to have had any influence.

The clinical picture is characterized by lack of appetite, more or less pronounced emaciation, light "secondary" anemia; sometimes slight elevation of temperature and tenacious nightly attacks of coughing coupled with attacks of asthma.

We did not succeed in changing the periodicity of these attacks. In the muco-purulent sputa, which did not have a metallic taste, many eosinophilic leukocytes were found. The x-ray pictures in Cases 1 and 2 were identical: increased lung markings with miliary foci diffusely distributed in the different lobes; they did not present the density of the nodules in miliary tuberculosis. The peripheral lymph glands were enlarged. The spleen was enlarged in 3 out of 4 cases. The basal metabolic rate was normal.

The globulin content of the serum-protein was increased. Persistent hyperleukocytosis was twice observed; the numbers varying between 16,000 and 41,000 per cu. mm. The eosinophilic leukocytes were all of the mature type. No worms, worm-eggs or other parasites were found in the sputum. The clinical picture corresponds with the descriptions from British-India by Weingarten (11) (81 cases), Treu (15), Vaidya (16) and Emerson (17).

Weingarten has given the name of "Tropical Eosinophilia" to this syndrome. As far as we could ascertain no mention is made of enlargement of peripheral lymph glands.

The patient of Emerson was first operated for a liver abscess. The amebiasis as etiologic factor cannot be excluded in this case, the more so as Stefano (18), Hoff and Hicks (19) have already reported the occurrence of transitory pulmonary infiltrations, accompanied by attacks of asthma, associated with amebiasis.

The cases of Valledor et al. from Habana and of Bass from the United States (one of the children came from Puerto Rico in the West-Indies) probably belong to the same category. The histological findings of Valledor resemble these of our cases.

The cases present great diagnostic difficulties. Many patients are sent to a specialist with the diagnosis of asthma, so that in regions where filariasis does occur, this diagnosis must be considered with suspicion. A diagnosis of Hodgkin's disease, in which enlargement of peripheral lymph glands can be combined with hypereosinophilia (adenie prurigene eosinophilique of the French authors) can be excluded by histological examination of an excised lymph gland and by the observation of the clinical course. Without a biopsy the differential diagnosis can be very difficult. It should not be forgotten that even the histological diagnosis may offer great difficulties (Major and Leger) (20).

In Case 2 eosinophilic leukemia could be excluded easily. In Loeffler's syndrome the principal symptom, the infiltration of the lungs, is of a more

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HETEROPHILE AGGLUTININS AND COLD AUTOHEMAGGLUTININS IN SCHISTOSOMIASIS, FILARIASIS, MALARIA, AND LEPROSY

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Whether heterophile agglutinins and cold autohemagglutinins develop in diseases such as schistosomiasis, filariasis, malaria and leprosy, is of practical and theoretical interest. Heterophile agglutinin determinations assumed practical clinical significance following the discovery by Davidsohn (1) of elevated titers in serum sickness and the finding of elevated titers in infectious mononucleosis by Paul and Bunnell (2). In infectious mononucleosis the determination is an accepted diagnostic procedure. More recently, elevated sheep-cell agglutinin titers have been reported in various bacterial diseases, in measles, filariasis, and in patients treated with parenteral liver (3–5). At the present time when the armed forces are of necessity exposed to the so-called tropical diseases, the possible occurrence of sheep-cell agglutinins in titers of clinical significance in filariasis and other parasitic diseases becomes important.

Heterophile antigens have been shown to be present in numerous bacteria, in higher plants, and in various animal species. The presence of such antigens in helminth parasites has only recently received attention. Mauss (6) studied rabbits infected with the nematode, *Trichinella spiralis* and demonstrated that rabbit serum which contained anti-trichinella antibody lysed sheep cells and that the hemolysin was absorbed by guinea-pig kidney emulsion. However, Rose (7) was unable to demonstrate significantly elevated titers of sheep-cell agglutinins and hemolysins in the serums from 17 human cases of trichinosis and from two experimentally infected rabbits. In the present study, search was made for evidence indicating that the nematode, *Wuchereria bancrofti*, and the trematode, *Schistosoma mansoni*, contain a heterophile antigen.

Recently Peterson et al. (8) and Turner (9) independently reported the occurrence of cold autohemagglutinins in high titer in certain cases of primary atypical pneumonia of unknown etiology. This finding has been confirmed by other workers (10) and may prove to be extremely useful as an aid in the sub-division of that part of the pneumonia complex for which no specific etiological agent has yet been established. Shone and Passmore (11) reported the presence of autohemagglutination in cases of pneumonitis and in addition noted that cases of chronic malaria and pulmonary tuberculosis frequently showed this phenomenon. In as much as African trypanosomiasis is the only other disease in which cold autohemagglutinins are known to occur consistently (12), a search for their presence in other parasitic diseases is indicated. For a general review of the subject of cold hemagglutination reference should be made to the recent publication by Stats and Wasserman (13).

I. METHODS

Venous blood specimens were collected in tubes containing sodium citrate. The majority of the specimens were kept at room temperature (27–33°C.) and were examined within 24 hours. A few specimens were examined 48 to 72 hours after being collected; these were stored in a refrigerator at 5°C. until used.

A. *Heterophile agglutinins:* The following technique, which is essentially the same as that used by Paul and Bunnell (2), was employed. Plasma was removed after centrifugation and inactivated for 20 minutes at 56°C. Starting with a plasma dilution of 1:4, serial doubling dilutions were made in Kahn tubes containing 0.5 ml. of normal saline solution. To each tube was added 0.5 ml. of a 2% suspension of thrice washed sheep red cells, followed by 1 ml. of saline solution. This gave a final concentration of 0.5% sheep cells in a total volume of 2.0 ml. with final plasma dilutions of 1:16, 1:32, 1:64, etc. The tubes were shaken, incubated for 1 hour at 37°C., and then placed in a refrigerator overnight at 5°C. On removal from the refrigerator, each tube was quickly inverted three times and examined for agglutination of the cells. The highest dilution of plasma showing

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macroscopic agglutination was considered to be the end point.

B. *Cold autohemagglutinins:* In testing for cold autohemagglutinins, the patient's cells were used with serial dilutions of the patient's plasma according to the following technique. The specimens of citrated blood were incubated for one hour at 37°C. (if specimens had been refrigerated, the incubation period was at least three hours). Plasma and cells were separated by centrifugation. A 1% suspension of washed erythrocytes was made in normal saline. Starting with a plasma dilution of 1:5, serial doubling dilutions were made in Kahn tubes containing 0.5 ml. of normal saline. Then 0.5 ml. of the suspension of the patient's cells was added, the tubes shaken, and placed in the refrigerator overnight at 5°C. This method gave a final concentration of 0.5% patient's cells in a total volume of 1 ml., with plasma dilutions of 1:10, 1:20, 1:40, etc. On removal from the refrigerator, each tube was inverted three times and the titer read as in the heterophile antibody tests. If positive, the tubes were reincubated for 1 hour at 37°C. and then reread.

II. HETEROPHILE AGGLUTININS: MATERIALS AND RESULTS

A. *Control group:* Heterophile agglutinin titers were determined on 273 Kahn-positive routine serums submitted to the Antilles Department Medical Laboratory. Of these, 74 were from continental soldiers (soldiers from the United States stationed in Puerto Rico) and 199 were from insular (Puerto Rican) troops. Certain of the serums were stored in the refrigerator for periods up to seven days before being examined. The results are summarized in table 1. (Note: In table 1 and figure 1 the "0" group includes possible titers up to 1:16, in as much as a 1:16 dilution of plasma was the lowest tested.) The Kahn-positive specimens are termed the "control" group, but it should be noted that serum specimens instead of plasma specimens were used and therefore the results may not be directly comparable. Also it is quite probable that the serums tested in the insular group included some from individuals with sub-clinical cases of schistosomiasis and filariasis since recent studies have shown that approximately 10% of the local registrants for Selective Service have schistosome ova in their stools (14) and in approximately 2.5% circulating microfilariae have been demonstrated (15).

B. *Schistosomiasis:* The material examined consisted of blood specimens from three groups of cases of schistosomiasis and from a small group of experimentally infected animals. The first group was composed of specimens from 108 Puerto Rican Selective Service registrants who were found to have ova of *Schistosoma mansoni* in their stools (14). The registrants had passed their final army physical examination and had what might be termed "subclinical infections," although some of the fecal specimens contained large numbers of ova. A second group consisted of five cases of schistosomiasis on the wards of the University Hospital of the School of Tropical Medicine; these had splenomegaly and hepatomegaly, and two showed

TABLE 1

Heterophile Agglutinin Titers

TYPE OF CASE	NUMBER OF CASES	NUMBER SHOWING TITER				
		0*	1:16	1:32	1:64	1:128
Control group						
Continental	74	63	8	2	1	0
Insular	199	130	42	19	4	4
Schistosomiasis						
Subclinical cases—registrants	108	89	11	7	1	0
Hospital cases	5	0	1	1	3	0
Ambulatory cases	10	5	1	2	1	1
Filariasis						
Subclinical cases—registrants	101	65	15	9	12	0
Clinical cases with microfilariae	3	2	0	0	1	0
Lymphangitis; no microfilariae	8	1	4	1	1	1
Malaria						
Vivax: Primary	17	10	3	3	1	0
Vivax: Reinfection or relapse	16	11	2	2	1	0
Falciparum: Primary	4	4	0	0	0	0
Falciparum: Reinfection or relapse	3	2	1	0	0	0
Leprosy						
Lepromatous	11	5	0	2	2	2
Neural	*7*	*4*	*0*	*1*	*0*	*2*

* Figures in the column headed "O" in tables 1 and 2 indicate the number of specimens showing no agglutination in the dilutions tested.

marked ascites. The third group consisted of ten ambulatory patients with stools positive for schistosome ova. These patients were receiving fuadin treatment at the same hospital. The results of these examinations are summarized in table 1.

Search was made for sheep-cell agglutinins in a small group of experimentally infected rabbits (16). Six adult rabbits were each exposed to water containing approximately 5,000 cercariae of *S. mansoni* for a period of one hour by partially submerging them in bell jars; control serums collected from each rabbit at this time showed no sheep-cell agglutinins. The rabbits were bled on the 9th, 17th, 25th, 35th, and 50th days after exposure. One rabbit died on the 45th day of the experiment and autopsy revealed developing schistosomes. The remaining animals were sacrificed on the 50th day and four of the five had schistosomes in the liver and mesenteric vessels. The serums were usually examined the same day the rabbits were bled using the technique outlined above; however, the initial dilution was 1:8, instead of 1:16. All of the specimens were negative for sheep-cell agglutinins.

C. *Filariasis:* Three groups of patients were studied. Of these, 101 specimens were from Puerto Rican registrants for Selective Service who had been found to have circulating microfilariae (15). These registrants had passed their final army physical examination and were considered to have a subclinical *Wuchereria bancrofti* infection. Three patients on the wards of the University Hospital of the School of Tropical Medicine in whom circulating microfilariae had been demonstrated and who showed one or more manifestations of filariasis, including chyluria, hydrocele, and femoral lymphvaricoceles, were examined. A third group of 8 individuals was studied in the filariasis clinic of the same hospital; this group

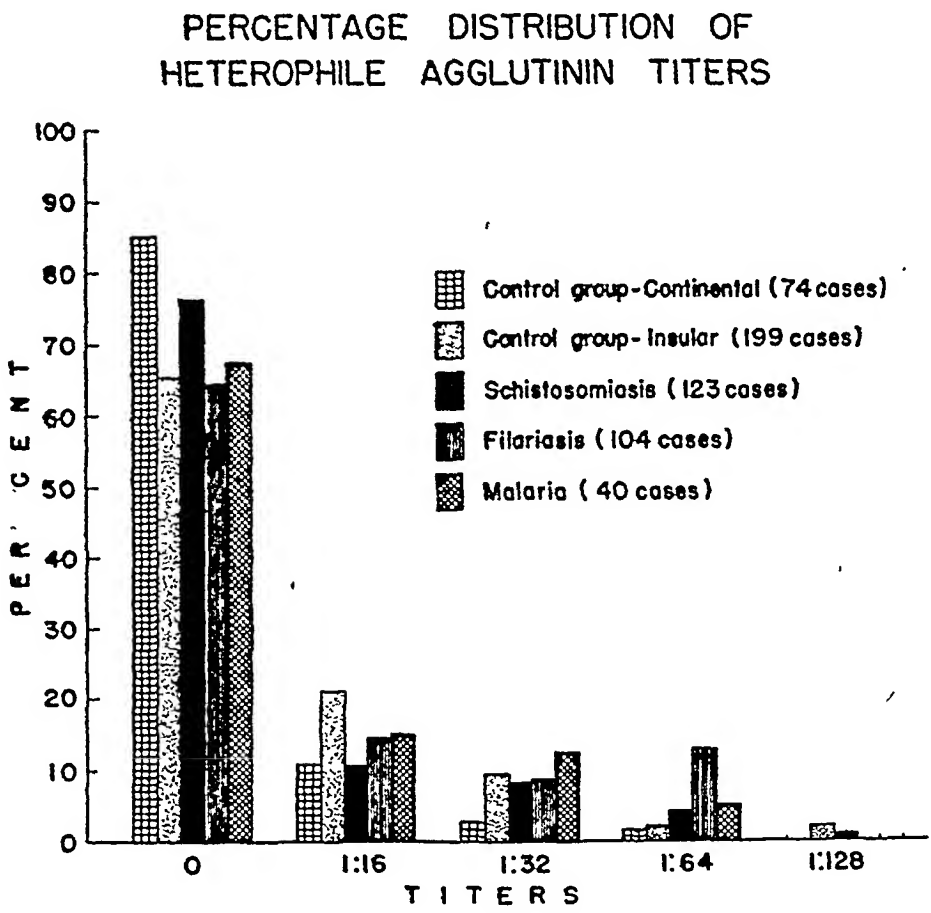

FIG. 1. PERCENTAGE DISTRIBUTION OF HETEROPHILE AGGLUTININ TITERS

of patients gave a history of repeated attacks of lymphangitis and several had marked elephantiasis, but in none had microfilariae been found and the group is therefore not classed with the other filariasis cases. The findings are summarized in table 1.

To determine the effect of prolonged refrigeration at 5°C. the twelve specimens of plasma from registrants that had a sheep-cell agglutinin titer of 1:64 were re-examined after being stored for five months. One showed a titer of 1:128, three had a titer of 1:64, three had a titer of 1:32, two of 1:16, and three were negative.

D. *Malaria:* Forty cases of malaria at the Station Hospital, San Juan, Puerto Rico, were investigated. In this group of cases the blood was obtained shortly after admission and before therapy had been instituted. Table 1 summarizes the type of infection and the titers of sheep-cell agglutinins obtained.

A second group, composed of twelve cases, was bled on the day after each patient had completed a course of quinine sulfate treatment (10.8 grams distributed over a period of 9 days). Of the twelve specimens, five showed no agglutinins, four had a titer of 1:32, and three a titer of 1:64. In nine of these cases control bloods were taken before therapy was started. Six of the cases had the same titer before quinine treatment as they had after treatment; two cases showed a rise in titer from 1:16 to 1:32 and one case rose from 1:16 to 1:64.

E. *Leprosy:* Eighteen lepers were studied at the Insular Leper Colony, Trujillo Alto, Puerto Rico. The cases are grouped as to the predominant type of leprosy and the heterophile agglutinin titers obtained in these groups are presented in table 1. Certain of the patients had received therapeutic diphtheria toxin or toxoid within the preceding twelve months. Of those receiving this treatment, 7 showed no agglutinins, and two each showed titers of 1:32, 1:64, and 1:128. In the untreated group, two were negative, one had a titer of 1:32, and two a titer of 1:128.

To determine the effect of storage at 5°C. on the heterophile agglutinin titer, three of the four plasma specimens which had a titer of 1:128 were re-examined after being stored for 21 days. They showed a fall in titer to 1:16, 1:32, and 1:64 respectively. The titer of the fourth specimen on re-examination after being stored 15 days was unchanged.

Rubino (17) noted that serum from lepers would cause an "agglutino-sedimentation" of formalized sheep erythrocytes; this reaction was considered to be specific for leprosy. Other authors (18) have confirmed his findings. Rubino's final modification of the method included an absorption with fresh sheep cells to remove hetero-agglutinins. However, this work suggested that it would be of interest to determine if serums with elevated heterophile agglutinin titers from various diseases, would agglutinate normal and formalized sheep cells in the same dilutions. Sheep cells were fixed in a final concentration of 10% formalin and then washed as described by Rubino. Heterophile agglutinin tests and a duplicate series of tests in which formalized sheep cells were substituted for fresh sheep cells, were then set up with serum or plasma from cases of leprosy, filariasis, schistosomiasis, malaria, and infectious mononucleosis. The fresh sheep cell series showed the following titers: four leprosy plasma specimens, titers 1:128, 1:64, 1:32, and 1:16; one filariasis plasma, titer 1:64; two schistosomiasis plasma specimens, titers 1:128, and 1:64; two malaria plasma specimens, titers 1:64 and 1:16; four infectious mononucleosis serums, titers 1:1024, 1:512, and two specimens, 1:256. In none of the serums or plasma specimens set up with formalized sheep cells was any agglutination apparent.

III. COLD AUTOHEMAGGLUTININS: MATERIALS AND RESULTS

A search was made for cold autohemagglutinins in cases of schistosomiasis, filariasis, malaria, and leprosy utilizing the same blood specimens as were used in the heterophile agglutinin study; however, no control group was run. In the great majority of specimens no cold autohemagglutinins could be demonstrated. The results are summarized in table 2. (Note: In table 2, the "0" group includes possible titers up to 1:10, in as much as a 1:10 dilution of plasma was the lowest tested).

IV. DISCUSSION

For comparison, the percentage distribution of the heterophile agglutinin titers obtained in the control groups, and in the proven cases of schistosomiasis, filariasis, and malaria is summarized in figure 1. In none of the patients studied were high heterophile agglutinin titers obtained, and the data indicate that heterophile antibody titers of clinical significance are not frequent in schisto-

somiasis, filariasis, and malaria. In a group of malaria cases, quinine treatment produced no significant increase in titer. Four of 18 specimens from lepers had a heterophile agglutinin titer of 1:128; because of the small number of specimens examined no conclusions can be drawn as to the significance of this finding. It is of interest that the continental group showed a lower titer of sheep-cell agglutinins than did the other groups, which were composed almost exclusively of insular personnel.

TABLE 2

Cold Autohemagglutinin Titers

TYPE OF CASE	NUMBER OF CASES	NUMBER SHOWING TITER				
		0*	1:10	1:20	1:40	1:80
Schistosomiasis						
Subclinical cases—registrants	108	104	1	2	0	1
Hospital cases	5	5	0	0	0	0
Ambulatory cases	10	10	0	0	0	0
Filariasis						
Subclinical cases—registrants	101	98	1	0	2	0
Clinical cases with microfilariae	3	3	0	0	0	0
Lymphangitis; no microfilariae	8	8	0	0	0	0
Malaria						
Vivax: Primary	17	15	2	0	0	0
Vivax: Reinfection or relapse	16	14	0	0	2	0
Falciparum: Primary	4	4	0	0	0	0
Falciparum: Reinfection or relapse	3	3	0	0	0	0
Leprosy						
Lepromatous	11	8	2	1	0	0
Neural	7	7	0	0	0	0

*Figures in the column headed "O" in tables 1 and 2 indicate the number of specimens showing no agglutination in the dilutions tested.

The non-identity of the sheep-cell agglutinins normally present in human serums, in serum sickness, and in infectious mononucleosis is generally accepted and differential absorption tests are in use clinically. In infectious mononucleosis, the agglutinin titer remains unchanged in serum stored at icebox temperatures (4, 19). The fact that some types of heterophile agglutinins are labile at 5°C. does not appear to be generally known. Bornstein (3) described a case of *Escherichia coli* bacteremia which developed a high heterophile antibody titer, and demonstrated that the strain of *E. coli* contained a heterophile antigen; the heterophile antibody titer in his case diminished during storage in the icebox. A similar fall in titer was noted in the present study in certain of the filariasis and leprosy bloods. Serums from four cases of infectious mononucleosis diagnosed locally showed no change in titer on storage for periods up to eight weeks.

From the results obtained no conclusions may be reached as to whether *W. bancrofti* and *S. mansoni* contain a heterophile antigen which at some stage in the disease process might produce significant antibody titers. It is possible that a titer of 1:64 in the 13 filariasis plasma specimens might represent such a stage of activity. Rabbits experimentally infected with *S. mansoni* showed no heterophile agglutinins.

It was noted that formalized sheep cells were not agglutinated by serums which agglutinated normal sheep cells in high dilution.

Cold autohemagglutinins were not demonstrated consistently in any of the diseases studied. This finding does not necessarily conflict with the report of Shone and Passmore (11) in as much as they worked with undiluted serum specimens and in the present study the initial dilution of plasma was 1:10.

V. SUMMARY

Heterophile agglutinin and cold autohemagglutinin titers were determined in 123 cases of schistosomiasis, 104 cases of filariasis, 40 untreated cases of malaria, and 18 cases of leprosy. In schistosomiasis, 4.9% had heterophile agglutinin titers over 1:32, in filariasis 13% had titers above 1:32, and of the malaria cases 5% had titers above 1:32; in no case was the titer higher than 1:128. In the small group of blood specimens from lepers, four showed a heterophile agglutinin titer of 1:128. Of twelve quinine-treated malaria cases, nine had a titer of 1:32 or lower, and three, a titer of 1:64.

Of the above group of 285 blood specimens, cold autohemagglutinins in a titer of 1:10 or higher could not be demonstrated in 279 or 97%. The few positive specimens showed no particular distribution and the highest titer noted was 1:80.

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LOCALIZATION OF TRIVALENT RADIOACTIVE ANTIMONY FOLLOWING INTRAVENOUS ADMINISTRATION TO DOGS INFECTED WITH DIROFILARIA IMMITIS[1]

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Most of the compounds which have shown any promise in the treatment of filariasis and some other parasitic diseases of man have been derivatives of antimony. Dogs infected with the heartworm, *Dirofilaria immitis*, and cotton rats infected with *Litomosoides carinii*, a nematode found in the pleural cavity, have been used in experiments to develop new chemotherapeutic agents for filariasis. Although useful to a certain extent, experiments of this sort have the serious limitation of failing to indicate the therapeutic value that may be expected in the treatment of infections in which the adult filariids are located in the lymphoid or dermal tissues, as is the case with *Wuchereria bancrofti* and *Onchocerca volvulus* infections in man. Since previously untested antimony compounds may prove of value in these and other parasitic infections, a reliable method of determining the distribution of antimony in the tissues would be of value in the further screening of compounds as well as in indicating the mechanism of toxic action.

The chemical determination of small amounts of antimony in body tissues and fluids has been generally unsatisfactory although a number of investigators have been able to measure the antimony content of metabolites with reasonable accuracy following treatment with antimonials. A review of these investigations has been presented by Goodwin and Page (1). The latter authors (1, 2) investigated the metabolism of antimony by the use of a polarograph and were able to determine the rates of excretion for a number of trivalent and pentavalent antimony compounds and to measure the antimony content of blood for a period of three hours after the injection. These workers also demonstrated the presence of trivalent antimony in the liver of the rabbit and rat after treatment with pentavalent antimony compounds.

With the aid of Doctor Roy Hertz of the Division of Physiology, we attempted to find a method for the biological assay of antimony by adding known amounts of antimony compounds to cultures of *Lactobacillus arabinosus* and *Saccharomyces cerevisae* to determine if small quantities of this element would inhibit the growth of these organisms. It was found that amounts of antimony added as anthiomaline in quantities up to 0.5 mgm. of antimony per culture exhibited no inhibitory effects on growth.

Experiments were next conducted to determine the possibility of the use of spectrographic analysis for quantitative estimation of antimony in the blood. This method did not have the required sensitivity because of the interference arising from the iron in the blood. Serum examinations obviated the above difficulty but left doubts as to whether all of the antimony was being determined. Even this method was not accurate when the antimony level was below 5 micrograms per milliliter.

It appeared that an extremely delicate method would be required in order to determine the blood and tissue distribution of antimony compounds. Compounds synthesized with radioactive antimony were then used to follow the fate of antimony in the animal body since this method appeared to offer most promise of any available at the present time.

Within the last few years, the use of artificially produced radioactive compounds has been increasing in experimental biology. Radioactive isotopes of an element have the same chemical properties as the stable isotopes of that element. The physical properties are changed, however, in such a manner that extremely minute amounts can be detected. This has led to the use of radioactive substances in physiologic work where such material

[1] Read at the Fortieth Annual Meeting of the American Society of Tropical Medicine, St. Louis, Mo., November 13–16, 1944.

can be administered and its course in the body followed. In addition to accurate quantitative detection, another technique is available for such studies wherein thin tissue sections are mounted on photographic plates, the radioactive materials producing exposure of adjacent areas of the plates, thus demonstrating the particular part of an organ containing the compound. Radioactivity may cause injury to tissues but no appreciable physiologic effect is ordinarily produced with the intensities required for tracing.

McCoy and Downing (4) and McCoy, Downing, and VanVoorhis (5) were the first to use radioactive tracers in parasitology. They were able to demonstrate by the use of radioactive phosphorus that *Trichinella spiralis* larvae in the encysted stage exchange ions with the host through the cyst wall and therefore probably carry on active metabolism.

EXPERIMENTAL PROCEDURE

Radioactive antimony was prepared by the bombardment of antimony with deuterons in the cyclotron of the Carnegie Institution of Washington. The radioactive antimony was chemically separated from other elements of the target and was recovered as nearly pure antimony trioxide. The spectroscopic examination of this purified product showed only traces of other metals. The antimony trioxide was then synthesized into the desired compound for the injection of animals. In these experiments, tartar emetic and sodium antimonyl xylitol were prepared by methods described elsewhere (3). Solutions of tartar emetic and sodium antimonyl xylitol containing 10 mgm. of antimony per milliliter were prepared for administration. The antimony trioxide was administered as an aqueous suspension.

The degree of radioactivity of the compounds and tissues was determined by means of Geiger-Mueller counters with scaling circuits and by use of the ionization chamber.

Dogs naturally infected with the heart worm, *Dirofilaria immitis*, were used throughout these experiments. The compound containing radioactive antimony was injected intravenously and blood samples were taken at intervals of 15, 30, and 45 minutes, and 1, 2, 4, 8, 16, 24, and 36 hours. To these blood samples dry sodium citrate was added in the proportion of 10 mgm. per milliliter. During the 36 hours after injection, the urine was collected at each 6 hour interval. At the end of 36 hours, the dogs were sacrificed by electrocution or by the intravenous injection of sodium pentabarbitol. The animals were then autopsied, the residual urine in the bladder collected, and samples of tissues were removed and placed in weighing dishes and covered.

Tissues were weighed quickly after their removal, placed in a desiccator containing phosphorus pentoxide, and kept under reduced pressure by means of a vacuum pump. After 16 hours of drying, the tissues were reweighed and the amount of weight loss determined.

Determinations of the antimony content of the tissues of the dogs injected with the radioactive antimony compounds were made by measuring the number of disintegrations per second per gram of tissue and comparing this with a known standard. This standard was made by adding to a sample of normal blood a known amount of radioactive antimony. Comparison of results obtained from such blood standards and standards prepared from aqueous solutions of the radioactive compounds showed that in the range of 0.25 to 40 micrograms of antimony the maximum deviation was $\pm$ 3 per cent with an average deviation of 1 per cent.

RESULTS OF DETERMINATIONS

Each of four infected dogs was treated with 0.8 mgm. of antimony per kilogram of body weight injected in the form of tartar emetic. The average rate of disappearance of the antimony from the blood is shown in figure 1. It will be noted that the antimony content is expressed in micrograms per gram of blood. The figure shows that the antimony left the blood at a decreasing rate and most of it had left the blood within a few hours. A slight secondary rise of the blood level was found in three of the four dogs in the 24 and 36 hour specimens. This rise may be due to excretion of antimony in the bile with subsequent reabsorption through the intestine.

Of the four dogs receiving tartar emetic, 22, 15, 6, and 7 tissues, respectively, were examined for their antimony content. The average results of these examinations are presented in figure 2. The results are plotted on a logarithmic scale because of the range of the observations. The liver invariably contained the greatest amount of antimony, the average being 10.7 micrograms of antimony per gram with a range of 9.1 to 14.2. Next in antimony content were the thyroid and parathyroid which together contained one-third as much antimony per gram as did the liver, the average being

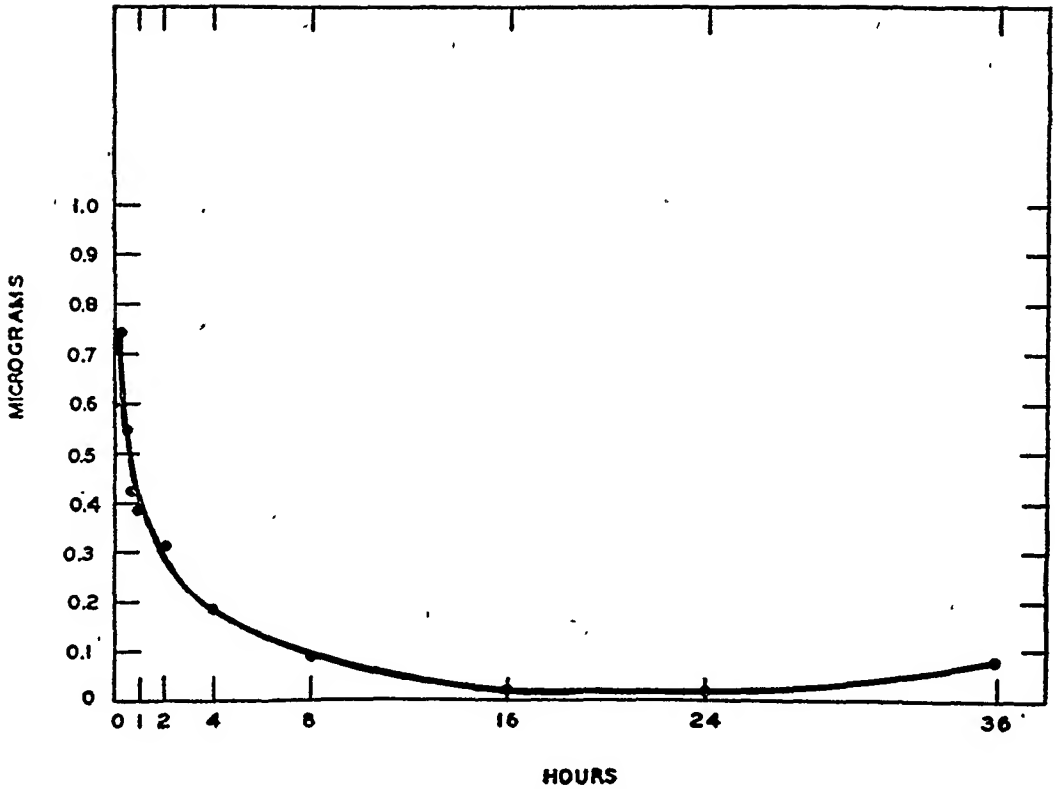

FIG. 1. Average micrograms of antimony per gram of blood of four dogs injected with 0.8 mgm. antimony as tartar emetic per kilogram of body weight.

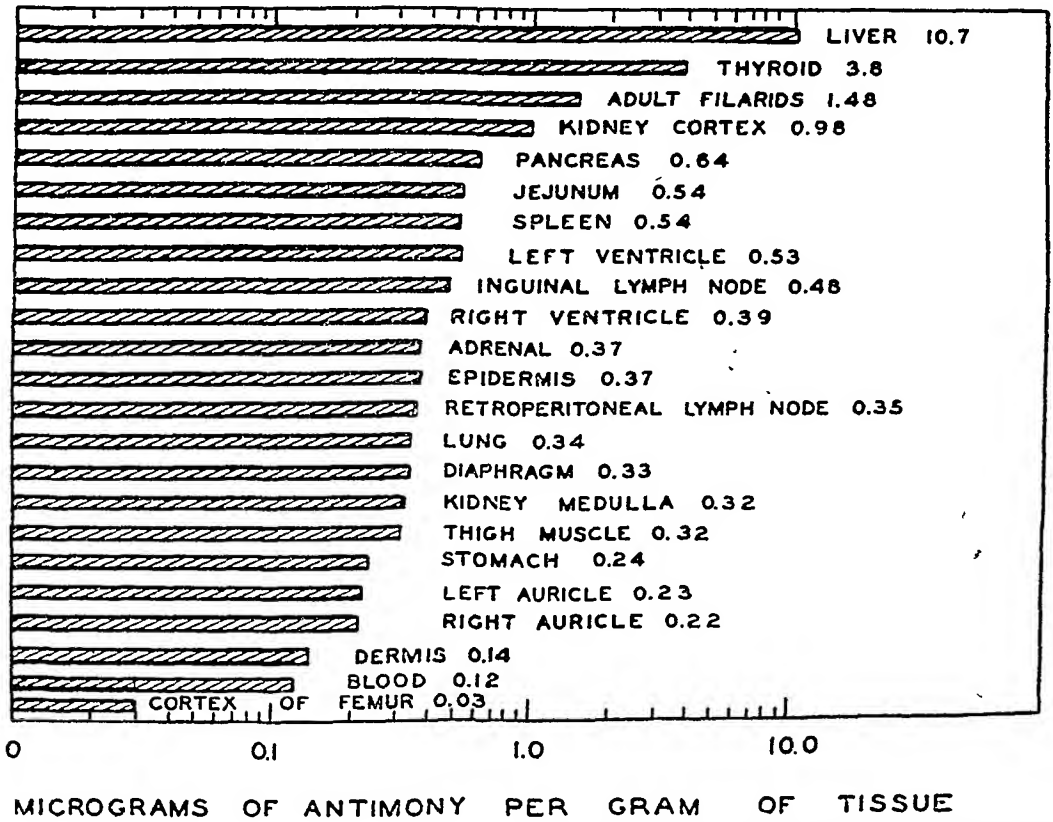

FIG. 2. Average micrograms of antimony in tissues at autopsy 36 hours after injection of dogs with 0.8 mgm. antimony as tartar emetic per kilogram of body weight.

3.8 micrograms of antimony with a range of 2.8 to 4.8. The adult *Dirofilaria immitis* recovered from the dogs contained about one-seventh and the kidney cortex one-tenth as much as the concentration in the liver. The figure lists a number of other tissues whose antimony content was so low that no appreciable concentration was indicated.

It is to be noted that lymph nodes, which in man are the site of predilection for *Wuchereria* adults and the dermis which is the site of predilection for *Onchocerca* adults apparently did not show a specific affinity for the antimony of tartar emetic.

Two dogs were injected with 0.8 mgm. of antimony per kilogram of body weight administered in the form of sodium antimonyl xylitol. The amount of antimony in the blood of both dogs was determined as was the amount of antimony in the liver of one dog at 36 hours. It was found that the blood level and liver content were approximately the same as those found in the dogs after the injection of tartar emetic.

The collection of six hour specimens of urine was unsatisfactory due to the irregular and infrequent urination of the dogs. However, the total urine collected over the interval, plus the residual urine expressed from the bladder at autopsy, contained all of the antimony excreted in the urine during this interval. The total amount of antimony excreted in the urine showed a great deal of variation. With the dogs treated with tartar emetic to give an antimony dose of 0.8 mgm. per kilogram of body weight, 9.9, 21.2, 4.0, and 21.2 per cent, respectively, of the total injected, was excreted in 36 hours. These amounts bore no direct relationship to the amount of antimony present in the liver, blood, thyroid, or the adult *Dirofilaria immitis* from the respective dogs. The urinary excretion of antimony in one dog injected with sodium antimonyl xylitol was 13.7 per cent in 36 hours.

A suspension of the relatively insoluble antimony trioxide was prepared from radioactive antimony and injected into one dog. In this animal it was found that the blood showed an almost complete absence of the compound as early as one-half hour after injection. Similarly the liver content and the urinary excretion rate were very much less than the figures noted following the injection of the soluble compounds mentioned above.

DISCUSSION

In the past, the use of artificially produced radioactive compounds has been limited almost entirely to studies of elements that are normally present in the body and mostly to the study of normal physiologic processes. It would appear that this method may have considerable value in toxicological studies of drugs that cannot be determined in small amounts in tissues by other means.

By the use of radioactive tracing techniques, we have been able to show the blood level and tissue distribution of tartar emetic injected intravenously into dogs in a single dose of 0.8 mgm. of antimony per kilogram of body weight. Calculations based on the antimony content of tissues removed from the four dogs 36 hours after the injection of tartar emetic showed that 39 to 48 per cent of the injected antimony had remained within the liver. The large amount of antimony accumulated in the liver at 36 hours after injection is not surprising because of the known fact that tartar emetic is likely to cause liver necrosis.

The large antimony content of the combined thyroid and parathyroid tissues was an entirely unexpected finding because to our knowledge there has been no laboratory or clinical indication that antimony affects either of these organs. This subject warrants further investigation and experiments are being undertaken to determine whether the high antimony content occurs in the thyroid alone or in both the thyroid and parathyroid glands.

The presence of antimony in adult *D. immitis* is of importance because the finding helps to explain the sterility of the female worms and the death of some of the adults under antimony therapy. In one of the four dogs treated with tartar emetic only one male worm was found. This parasite contained 0.6 microgram of antimony per gram as compared with the average of 1.8 micrograms of antimony per gram for both male and female parasites recovered from the other three dogs. Further work on the antimony content of the adult parasite is being carried on.

The moderate amount of antimony found in the kidney cortex may represent in part that deposited extracellularly by the urine. There was no specific uptake of the antimony of tartar emetic by lymph and dermal tissues. It is hoped that other compounds of antimony will be found to reach these tissues in higher concentrations.

Goodwin and Page (1) found that mice excreted 29.8 per cent and 34.8 per cent of intraperitoneally injected antimony in 24 and 48 hours, respectively, after the administration of tartar emetic with a dosage of 6 mgm. of antimony per kilogram of body

weight. Mice excreted 61.5 per cent and 68.5 per cent of intravenously injected antimony in 24 and 48 hours, respectively, after the administration as tartar emetic of 3.8 mgm. of antimony per kilogram of body weight. The lower excretion rate obtained in our experiments may be due to the use of a different experimental animal and a lower dosage rate.

In a previous paper (3) sodium antimonyl xylitol was found to be less than one-half as acutely toxic as was tartar emetic. The results of blood, liver, and urinary excretion examinations of dogs treated with both compounds fail to furnish a clue as to the reasons for this difference.

The suspension of antimony trioxide was used in these experiments because of our previous success in eliminating microfilariae from dogs with this compound. We had also observed that some of the less stable antimonyl compounds decomposed *in vitro* into antimony trioxide. It was thought that these compounds, although curative, may undergo some such decomposition in the body. The injection of the suspension was found to cause a much lower content of antimony in the liver and blood than did the soluble compounds. No evidence was therefore obtained as to the decomposition of the latter type of compound.

SUMMARY AND CONCLUSIONS

1. Dogs naturally infected with *Dirofilaria immitis* were treated with single intravenous injections of tartar emetic, sodium antimonyl xylitol, and an aqueous suspension of antimony trioxide each prepared from radioactive antimony. Animals were sacrificed 36 hours after the injection.

2. Quantitative estimations of the antimony present in the blood after the injection of tartar emetic and sodium antimonyl xylitol showed that there was an initial rapid decrease of the element in the blood during the first hour followed by a slow removal for the next 4 to 16 hours. In some cases, there was a slight secondary rise in the blood level at 24 or 36 hours.

3. Following the injection of tartar emetic, examination of the dry tissues from 22 organs revealed that the liver contained the largest amount of antimony. The combined thyroid and parathyroid tissues contained the next largest amount. The adult *Dirofilaria immitis* ranked third in antimony content. It is possible that these tissues have a specific affinity for antimony since their antimony content was considerably greater than could be expected as the result of a simple distribution of a soluble compound throughout body fluids.

4. The concentration of antimony in the dermal and lymphatic tissues was of very low degree. However, other compounds are under trial and it is possible that one or more of them will be found in greater concentration in these tissues and will thus offer more promise in the treatment of such diseases as the filariases caused by *Wuchereria* spp. and *Onchocerca volvulus*.

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COMPARATIVE YIELDS OF ENDAMOEBA HISTOLYTICA-ORGANISM *t* FROM SOLUBLE AND INSOLUBLE INGREDIENTS OF EGG WHITE IN FRESHLY PREPARED AND STORED MEDIUM[1]

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The nature of the problem of determining the nutritive requirements of *Endamoeba histolytica* is well known having been discussed by a number of workers in this field including Boeck and Drbohlav (1), Dobell and Laidlaw (2), Cleveland and Collier (3), Rees, Reardon, and Jacobs (4), Chinn, Jacobs, Reardon, and Rees (5), Snyder and Meleney (6), Rees, Bozicevich, Reardon, and Daft (7), and others. The earlier workers used a base of heat coagulated protein with an overlay containing non-coagulated protein and non-hydrolyzed starch and noted that the amoebae ingest the starch. Frye and Meleney (8) and Reardon and Rees (9) have determined that the thermolabile ingredient is not required. The former workers substituted heat sterilized liver extract and the latter dispensed with the thermolabile ingredient without any substitution. It does not follow, however, that *E. histolytica* utilizes ingredients as they occur in sterile medium because growth thus far has not occurred without an associated flora [Cleveland and Sanders (10), Meleney, Frye, Leathers, and Snyder (11), and Snyder and Meleney (6)]. Dobell and Laidlaw (2) stated that *E. histolytica* depends on the flora for its food supply. More recently, Snyder and Meleney (6) suggested that the bacteria may furnish suitable conditions for the respiration of the amoebae. Specifically they stated that "the association of *E. histolytica* with bacteria is functional rather than merely coincidental." It appears, therefore, that in the presence of a mixed flora of undetermined species of bacteria the problem of ascertaining the specific nutritive requirements of *E. histolytica* is insurmountable. This fact was recognized by Dobell and Laidlaw (2) in the following statement: "Cultivation of amoebae by methods involving such a number of unknown variables . . . is obviously unscientific."

We have, therefore, approached the problem by establishing the amoebae in culture with a single species of bacteria and have determined that this species, designated as organism *t*, is micro-aerophilic and utilizes dextrose and probably other reducing substances of the medium with the production of hydrogen and thus establishes an anaerobic environment favorable for the growth of the amoebae [von Brand, Rees, Jacobs, and Reardon (12)]. The relative growth rates of *E. histolytica* and organism *t* have been investigated (7) in various media with the result that very high yields of amoebae have been obtained from a simplified L.E.S. medium prepared from egg white enriched with vitamins and cholesterol, substances which are lacking in egg white though present in whole egg. However, contrary to expectations we have found (7) that medium prepared from egg yolk did not support growth of the amoebae and, as expected, that the enrichment of yolk medium with vitamins and cholesterol was ineffective. It appears then that heat coagulated egg white contains growth factors for *E. histolytica* that are lacking in the vitamin-cholesterol rich yolk. Our data show further (7) that without cholesterol the vitamin enriched egg white will not support the growth of the amoeba and data of Rees and Reardon (13) show that although organism *t* requires the vitamins it grows as well without as with cholesterol. In vitamin-cholesterol enriched media prepared from 10 animal and 3 plant peptones there was good growth of organism *t* and practically no growth of amoebae. On the other hand, in a number of media prepared from papain digests of peanut, soy bean, cotton seed, rice, liver, whole egg, and egg white [method of Brewer (14)] there was occasional good growth of amoebae and uniformly excellent growth of organism *t* [Rees and Reardon (13)]. Numerous attempts over a period of 6 months to make papain digests that were uniformly good for

[1] Read at the Fortieth Annual Meeting of the American Society of Tropical Medicine, at St. Louis, Mo., November 13–16, 1944.

[2] With the collaboration of Principal Biochemist Floyd S. Daft of the Division of Physiology.

growth of amoebae were unsuccessful [Rees and Reardon (13)]. It appears then that cholesterol is a growth factor for the amoeba and not for organism *t*. Also that in the absence of the requisite basic materials amoeba nutrients are not furnished by organism *t*. The latter occur in egg white and as indicated above our efforts to find a substitute have failed. In the present work we have sought to determine whether nutrient materials for *E. histolytica*-organism *t* are localized in soluble or insoluble fractions of coagulated egg or egg white and also to what extent the amoeba nutrients of egg white medium are affected by oxygenation.

EXPERIMENTAL DATA

The methods described by Rees, Bozicevich, Reardon, and Daft (7) for obtaining quantitative data on the growth of *E. histolytica* in Florence flasks were used in the present work. In addition, Rees and Reardon (13) have devised a method of measuring gas produced by organism *t* in the flask cultures and the figures thus obtained serve as an index of the growth of organism *t* under these conditions since no gas is produced by the amoebae [von Brand, Rees, Jacobs and Reardon (12)]. Details not previously published are as follows: A gas measuring cylinder is made by fusing a piece of 9 mm. glass tubing into the bottom of a 100 ml. test tube with the tubing inserted through a rubber stopper, by means of which the cylinder is held in position in the culture flask with the tubing end extending almost to the bottom of the overlay. The gas produced during incubation displaces an equivalent amount of overlay and the latter is measured in the test tube end of the cylinder. The figures for organism *t*-growth reported in the present paper are based on average measurements from 2 or more of a five flask set of medium. As in previous work (7), the figures for amoeba growth are based on average haemocytometer counts of two or more workers from pooled harvests of all 5 flasks.

A number of experiments were set up to ascertain whether amoeba nutrients released from egg medium might dialyze through a sausage casing membrane. Egg slants prepared in quart milk bottles were overlaid with Locke's solution containing rice flour and into this medium, following a method devised by Doctor J. H. Brewer, was suspended a loop of casing membrane filled with rice flour-enriched Locke's solution. In all cases sufficient length of membrane was used to permit a considerable segment to lie in contact with the egg base so that the oxygen potential occurring at this level might affect the casing contents. The loop was seeded with *E. histolytica*-organism *t*; the bottle medium with organism *t*. In exceptional cases as many as 2,500,000 amoebae were harvested per loop but in other cases the size of the harvest did not indicate any growth of amoebae. The causes of these variations have not been ascertained.

Our remaining experiments are summarized in tables 1 and 2. In the first series (table 1) a water insoluble fraction of heat coagulated egg white was separated from a water soluble fraction by centrifugation of Waring blender emulsions (table 1, W coag, W inf). The former fraction contained ovalbumin, probably some ovomucin, and all other egg white proteins except ovumucoid. The latter fraction contained ovumucoid and probably some ovumucin. From media prepared from each of the fractions and also from both fractions in the same medium (W ci) the yields of amoebae were far below those of the control indicating that the Waring blender treatment or some of the other manipulations may have affected amoeba producing properties of egg white. The experiments therefore failed to demonstrate whether the nutrients were localized in either fraction. From each fraction (W coag, W inf), the growth of organism *t* was lower than the control but from the combined fractions (W ci) as good as the control indicating that the manipulations *per se* had not adversely affected its growth factors.

In the second series of experiments (table 1, A coag, A inf) separation of the insoluble and soluble fractions was brought about by pouring the raw egg white into boiling Locke's solution and straining out the coagulum through cheese cloth. The average results on amoeba growth from the insoluble fraction medium (A coag) were about the same as from the Waring blender series (W coag). On the other hand, from the soluble fraction (A inf) and also from the combined fractions (A ci) the growth was nil. In all cases (A coag, A inf, A ci), the growth of organism *t* was about the same as in the control medium. We are unable to explain these discrepancies of amoeba growth between fractions of egg white presumably containing the same respective constituents but prepared by two different methods. In each case, organism *t* nutrients were far less sensitive to unknown factors inherent in the methods of procedure than were amoeba nutrients.

In a third series of experiments (table 1, A dis), the experimental medium differed from the control medium in only one respect, namely, that the coagulated egg white was dispersed in the overlay and not confined in a base. Excepting one experiment of this series, amoeba growth was nil; without exception the growth of organism *t* was as good as in the control. These results appear to point to some factor, probably an oxygen potential, that is operative only in a well defined egg white base.

the dissolved oxygen by boiling medium after storage had some restorative effect on amoeba producing properties (table 2, Cb). A stored base with fresh overlay was a deficient medium (table 2, C fO/B), but a stored overlay on a fresh base was a fair medium (table 2, C O fB) indicating that failure to produce amoebae was due to factors other than toxins. Cotton stoppered overlay used as a wholly liquid medium failed to produce amoebae (table 2, CO). Thus the data on the cotton stop-

TABLE 1

*Yields of Endamoeba histolytica-organism t in media prepared from fractions of heat coagulated egg white and from egg white of pullet eggs, all enriched with the vitamin-cholesterol formula F_5C, Rees, Bozicevich, Reardon and Daft (1944)**

KIND OF MEDIUM	WHOLLY LIQUID OR DIPHASIC wl or dp	NUMBER OF TESTS	YIELDS						CONTROL	
			E. histolytica			organism *t*			*E. histolytica*	organism *t*
			Maximum	Minimum	Average	Maximum	Minimum	Average	Average	Average
W coag	dp	2	41	39	40	35	29	32	139	66
W inf	wl	2	74	63	64	48	30	39	139	66
W ci	dp	2	90	39	65	67	68	68	139	66
A coag	dp	7	88	18	54	100	30	64	225	77
A inf	wl	7	12	4	6	110	32	63	225	77
A ci	dp	7	15	2	7	113	76	96	225	77
A dis	dp	6	213	4	53	121	52	75	232	82
AF_5CP	dp	2	424	397	411			72	-	

* The figures by 10,000 represent the number of amoebae per Florence flask culture. The figures for organism *t* represent ml. of gas per flask produced during the 72 hour period of incubation.

Key: W coag = water insoluble fraction of Waring blender emulsion of heat coagulated egg white.
W inf = infusion from centrifugalized Waring blender emulsion.
W ci = recombination of the above two fractions.
A coag = water insoluble fraction strained from egg white that was poured into boiling Locke's solution.
A inf = filtrate of the above.
A ci = recombination of the two fractions.
A dis = egg white coagulated by pouring 50 ml. amounts into Florence flasks containing 200 ml. of boiling Locke's solution.
AF_5CP = egg white medium from pullet eggs.

The last series of experiments of table 1 (AF_5CP) show unusually high yields of amoebae from pullet egg white. Untabulated data have shown high yields from spring and early summer eggs compared with those from fall and winter eggs. These differences of performance of egg white material are due to unknown factors.

The first series of experiments of table 2 (C) show marked loss of amoeba producing properties of cotton stoppered, stored medium, the loss increasing with the passage of time but not with indicated increase in temperature. Driving out

pered series indicate that loss of amoeba producing properties was probably due to oxygenation and that a fresh base functioned to produce a suitable oxygen potential.

In contrast with cotton stoppered medium, the loss of amoeba producing properties from rubber stoppered stored medium (table 2, R) was slight, especially when residual oxygen in the neck of the flask was absorbed by pyrogallic acid (table 2, Rpy). A similar contrast occurred between the amoeba yields of cotton stoppered and rubber stoppered stored base with fresh overlay and with

TABLE 2

*Yields of Endamoeba histolytica-organism t in stored egg white medium enriched with the vitamin-cholesterol formula F_5C, Rees, Bozicevich, Reardon and Daft (1944)**

KIND OF MEDIUM	TEMPERATURE DURING STORAGE	PERIOD OF STORAGE	NUMBER OF TESTS	YIELDS		CONTROL	
				E. histolytica	organism *t*	*E. histolytica*	organism *t*
				Average	Average	Average	Average
	°C.	days					
C	37	10–20	7	20	46	139	86
C	37	30–60	2	70	60	266	
C	10	10–20	4	82	43	196	68
C	10	30–60	2	5	48	249	70
Cb	37	14–30	2	142	84	171	
Cb	10	10–20	2	96	98	181	
Cb	10	30–60	2	74	51	172	40
C fO/B	37	10–20	7	35	16	174	64
C fO/B	37	31	1	86	38		
C fO/B	10	10–20	3	106	51	215	64
C O/fB	37	15	1	186		266	
C O/fB	10	16	1	149		171	
CO	37	10–20	6	21		134	
CO	10	10–30	3	44	71	181	
COb	37	16	1	132		171	
COb	10	10–30	4	94	89	181	
R	10	10–20	4	173	60	235	
R	10	30–60	3	174	60	317	90
Rb	10	10–20	2	289	104	295	64
Rb	10	30–60	2	172	81		
R fO/B	10	10–20	2	155	48	190	
R fO/B	10	30–60	2	81	24		
R O/fB	10	20–40	2	244	91	190	
RO	10	20–40	2	113	87		
Rpy	10	20–30	2	267	93	135	92
Rpy fO/B	10	20–30	2	196	85	135	92
Rpy O/fB	10	30	1	135	52		
RpyO	10	20–30	4	148	73		

* The figures by 10,000 represent the number of amoebae per Florence flask culture. The figures for organism *t* represent ml. of gas per flask produced during the 72 hour period of incubation.

Key: C = cotton stoppered stored medium.
Cb = same, boiled to drive out oxygen.
C fO/B = cotton stoppered stored medium, overlay poured off, fresh overlay added to base.
C O/fB = cotton stoppered stored overlay on fresh base.
CO = cotton stoppered stored overlay as a wholly liquid medium.
COb = same, boiled.
R = rubber stoppered stored medium.
Rb = same, boiled.
R fO/B = rubber stoppered stored medium, overlay poured off, fresh overlay added to base.
R O/fB = rubber stoppered stored overlay on fresh base.
RO = rubber stoppered stored overlay as wholly liquid medium.
Rpy = rubber stoppered stored medium with residual oxygen absorbed by pyrogallic acid.
Rpy fO/B = same, overlay poured off, fresh overlay added to base.
Rpy O/fB = stored overlay on fresh base.
RpyO = stored overlay as wholly liquid medium.

those of wholly liquid medium (table 2, R fO/B, R O/fB,RO). When oxygenation was adequately prevented with the use of pyrogallic acid (RpyO) the overlay as a monophasic medium produced a crop of amoebae comparing favorably with that of our control diphasic medium. The data in table 2 are all in agreement with the theory that oxygenation of stored medium may affect its amoeba producing properties. With several exceptions the growth of organism *t* was not appreciably affected by storage of the medium indicating that the factors exercised a specific effect for *E. histolytica.*

DISCUSSION

Our experiments with egg white fractions failed to indicate whether amoeba nutrients are localized in components of egg white, apparently because the methods employed were inadequate. On the other hand, our data on stored medium indicate that oxygen free stored overlay containing ovomucoid produced a good growth of amoebae. Good growth occurred also from the reoverlaid stored oxygen free base which had lost an undetermined amount of ovomucoid.

The data presented here lend support to a theory expressed by Rees, Reardon, and Jacobs (4) and also by Snyder and Meleney (6) that growth of *E. histolytica* is facilitated by a lowered oxygen potential. There appears to be some reducing action in a well defined egg white base that is operative only with rare exceptions in egg white coagulum dispersed in the overlay. From stored medium exposed to oxygenation this reducing power was lost but in oxygen free medium it was retained.

Our data on the growth of organism *t* are of interest primarily in demonstrating the extent to which we were dealing with amoeba nutrients rather than bacteria nutrients. Thus our method of isolating the amoeba with a single species of bacteria and obtaining quantitative data on the growth of both kinds of organisms may eventually lend itself to the attainment of our final goal, that of cultivating *E. histolytica* without bacteria.

SUMMARY

The growth rates of *Endamoeba histolytica* and organism *t* were measured in media containing fractions of egg white as well as in stored egg white medium and the data compared with those obtained with fresh egg white medium. All media were enriched with vitamins and cholesterol.

Water insoluble and water soluble fractions of heat coagulated egg white obtained by Waring blender emulsification of coagulated egg white and by pouring raw egg white in to boiling Locke's solution failed to nourish the amoebae but supplied nutrients for organism *t.*

A diphasic egg white medium with the coagulum dispersed in the overlay failed with one exception to produce amoebae but produced uniformly good growth of organism *t.*

In a limited number of experiments, pullet egg white and egg white from spring and early summer hen eggs produced better crops of amoebae than were obtained from fall and winter eggs.

Oxygenation occurring in stored cotton stoppered medium resulted in loss of amoeba-producing properties.

In rubber stoppered stored medium in which residual oxygen was absorbed by pyrogallic acid there was no apparent loss of amoeba producing properties.

Ovomucoid diffusing into the oxygen free overlay of stored egg white medium produced good growth of amoebae in a wholly liquid medium.

Support was furnished for the theory that fresh egg white base probably acting in conjunction with organism *t* is concerned in the oxygen potential of the medium.

The growth of organism *t* was not appreciably affected by factors operating in stored medium thus indicating that the factors concerned were specific for the amoebae.

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IMMUNITY REACTIONS IN EXPERIMENTAL RELAPSING FEVER[1]

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It is generally believed that the immunity which develops after an attack of relapsing fever is an "infection immunity." Persistence of spirochetes in the brain presumably accounts for this immunity which is usually of short duration, although opinions differ on the mechanism involved (1–4). Certain aspects of this problem have been studied in connection with the course and chemotherapy of a California strain of experimental relapsing fever infection in Chinese hamsters (*Cricetulus griseus*). During these studies Chen (5, 6) found that spirochetes could be demonstrated in the brains of treated and untreated Chinese hamsters as long as 60 days after their disappearance from the peripheral blood. All animals remained at least partially immune for as long as 43 days after the initial infection. This would suggest that residual infection plays an important rôle in this immunity reaction.

A continuation of these studies is the subject of the present report. Two *Macacus rhesus* monkeys were infected with the same California strain of *Spirochaeta recurrentis*. 0.02 cc. per kilogram of blood from infected Chinese hamsters was inoculated intraperitoneally. Both monkeys exhibited spirochetes in their peripheral blood 60 and 72 hours after inoculation and the blood remained positive for from 24 to 36 hours. Seven days after the disappearance of spirochetes from the blood, a second inoculation was made using twice the quantity of blood first given. Neither fever nor spirochetes were noted over a ten-day period. A third inoculation, this time subcutaneously, of a similar amount of blood from an infected mouse failed to produce any signs or symptoms of infection. These results would tend to support Coleman's opinion (3) that a very mild infection may produce definite immunity.

Twenty-eight and 33 days after the disappearance of spirochetes from the peripheral blood biopsies of the brain were performed. Specimens from each monkey exhibited spirochetes. These could be demonstrated by the use of Steiner's stain, in the grey matter and blood vessels of the brain (5).

Subsequent to biopsy each monkey was inoculated intraperitoneally with 2.0 cc. of blood from a patient suffering from the Chinese strain of relapsing fever infection in an effort to determine whether any cross-immunity existed between the tick-borne California strain and the louse-borne Chinese strain of *Spirochaeta recurrentis*. No spirochetes were observed in the peripheral blood of the monkeys during a 31 day period of observation although the blood was found to be infectious for hamsters. Tissue removed at a second biopsy of the brain of each monkey, 50 and 54 days after the first biopsies, revealed no spirochetes in the frontal lobe area.[3]

The results of these studies of material removed by biopsy of the brain would suggest that the residual spirochetes observed at the first biopsies had disappeared within 82 and 83 days after the peripheral blood had become free of spirochetes. The possibility remains that residual brain infection still existed although the number of spirochetes in the brain may have been too small to observe on microscopic examination.

In connection with studies of the development of immunity to homologous strains in hamsters (6, 7), four animals were splenectomized and inoculated intraperitoneally with 0.2 cc. of blood from a patient suffering from the louse-borne Chinese strain of relapsing fever. Three of the four hamsters exhibited spirochetes in the peripheral blood four to six days subsequently. Twenty-three days later these hamsters were inoculated with blood from hamsters infected with the tick-borne California strain. One comparatively short period of

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[3] We are indebted to Dr. S. T. Kwan of the Department of Surgery for performing the biopsies.

spirochetemia resulted in contrast to the three or four attacks observed in control animals. This additional finding would tend to support the earlier observations in hamsters and monkeys. The brains of hamsters used in the last experiment were found to contain spirochetes.

Hamsters inoculated with brain tissue which contained spirochetes failed to produce active infection but when these same animals were subsequently inoculated with infected hamster blood spirochetemia developed. There was no appreciable difference in the infectiousness of hamster blood containing spirochetes which was mixed with brain tissue and kept for 22 hours at ice box temperature and infected hamster blood to which no brain tissue had been added.

SUMMARY

The evidence accumulated from studies of experimental relapsing fever in Chinese hamsters and in *Macacus rhesus* monkeys would indicate that cross-immunity exists between infections with the tick-borne California strain of *Spirochaeta recurrentis* and the louse-borne Chinese strain.

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STUDIES IN EXPERIMENTAL SYSTEMIC MYCOSIS

I. Systemic Chromomycosis (Chromoblastomycosis) in Mice: Preliminary Study

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The term chromoblastomycosis, as originally used by Terra and coworkers (1) to designate a fungus disease, was revised by Moore and de Almeida (2) to chromomycosis. The disease does not correspond to a true blastomycosis since the fungus divides, both in culture and in tissue, by cross wall or septum formation and not by budding. The newer term eliminates the possibility of confusion.

Numerous cases of chromomycosis have been reported throughout the world. The majority were caused by the fungus *Hormodendrum pedrosoi*, a few by the closely related *Phialophora verrucosa*.

The disease is rarely diagnosed clinically except in endemic areas, where its manifestations are generally considered to be largely limited to the skin of the lower extremities, especially of the feet and shanks. Lesions have, however, been described on the hand, ear, face, shoulder, arm, neck, buttocks, and thigh. It is the consensus that these are due to direct inoculation of the fungus into an area of local injury.

Metastases have been reported in only two cases: one from Algiers (3) states, "there was a gummoid mass in the quadriceps muscle." This may not represent chromomycosis, as the fungus isolated from it was not pigmented. The other case was studied by Carrion (4) in Puerto Rico. He described the initial lesion on the toe of the left foot. Distinct lesions were found in the lower third of the left forearm and in the middle third of the right thigh. That in the forearm was described as a "lumpy mass" measuring about three by one and one half inches. It was deep within the subcutaneous tissues and appeared adherent to the underlying bone. The thigh lesion was the size of a large bean and lay within the fibrous sheath of the rectus femoris muscle. The Hormodendrum was demonstrated in both of these lesions. The location of the masses and the absence of overlying skin changes led the author to believe he was dealing with metastases from the toe rather than with autoinoculation phenomena.

It is difficult to differentiate this disease microscopically from other skin granulomata, especially those of mycotic origin. A definite diagnosis can be established only by demonstrating the organism upon microscopic examination aided by cultural studies.

The cutaneous lesions of chromomycosis have certain characteristics which may serve to aid in the diagnosis of the disease. These were described by Moore et al. (5) thus: "The most pronounced changes are found in the dermis. Here one sees edema, a pronounced cellular infiltration and, in older lesions, evidence of fibrosis. The cellular infiltration is made up of polymorphonuclear leukocytes, lymphocytes, epithelioid cells, plasma cells, eosinophiles and Russel's fuchsin bodies, which have been noted in tissue sections by others. Giant cells of the foreign body or Langhans type may be present as well as macrophages. There is sometimes a tendency to pseudotubercle formation in the infiltrate. Fibroblastic changes may occur. Necrosis and microabscesses such as are found in Gilchrist's disease are not prominent in chromomycosis. The sclerotic cells of the fungus are found scattered throughout the corium, especially in the abscesses or in giant cells. They occur either as single cells or in mulberry-like clusters."

Attempts to produce chromomycosis in animals have been reported. Carrion and Koppisch (6) inoculated 22 animals with a culture of *Hormodendrum* isolated from a case of chromomycosis. Four monkeys and ten rabbits were injected by rubbing a suspension of the organisms into scarified skin. No lesions were seen in these animals after seven to ten months of observation. Eight white rats were inoculated as follows: 3 received 0.75 cc. of the suspension subcutaneously, while 5 were injected with 1.5 cc. intraperitoneally. One rat of the latter group died four and one half months later with

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extensive pneumonia but no gross mycotic lesions. The peritoneum was unaltered, and microscopic study of the liver revealed several round, sharply outlined nodules. The central areas in some consisted of dense, hyalinized fibrous tissue; in others, of less dense connective tissue and a few shrunken epithelioid cells. The Hormodendrum was found in a number of these lesions. In some the spores were seen within giant cells. No other animal showed infection after the same period (7–10 months).

TABLE 1

Schedule of Sacrifice and Results for mice inoculated intravenously

ANIMAL	SACRIFICED POST INOCULATION	LESIONS PRESENT
	days	
H1	7	Lung, kidney, liver
H2	7	Lung, kidney, liver
H3	15	Lung, kidney
H4	18	Lung, kidney
H5	27	Lung, kidney

Schedule of Sacrifice and Results for mice inoculated intraperitoneally

ANIMAL	SACRIFICED POST-INOCULATION	LESIONS PRESENT
	days	
HP1	7	Focal abscesses in the peritoneum of all animals
HP2	7	
HP3	11	
HP4	15	
HP5	18	

Gomes (7) inoculated intradermally one rabbit, four guinea pigs, and one rat. Three of the guinea pigs died on the sixteenth day following inoculation, "with multiple congestive phenomena." There were no granulomatous lesions or abscesses, and there was no report of histologic examination. The rabbit and rat showed a "papillomatous reaction" approximately 38 days after inoculation.

EXPERIMENTAL PROCEDURE

The organism, *Hormodendrum pedrosoi*, #8615, used in the following experiments, was supplied by Dr. Chester Emmons of the United States Public Health Service. It was grown for 96 hours on Sabouraud's dextrose liquid media. The surface growth was harvested, ground in a mortar with physiologic salt solution and a suspension made equal in density to a #4 McFarland barium sulfide nephlometer.

Five white mice were injected intraperitoneally with 0.4 cc. of the suspension, and five were injected intravenously with 0.2 cc. The mice were sacrificed (chloroform) at intervals up to the twenty-seventh post-inoculation day. Table I shows the schedule of sacrifice and results. Complete autopsies were performed on all animals, and sections of the lungs, liver, spleen, kidneys, and adrenal glands were made for microscopic study.

RESULTS

Gross pathology

The mice injected intravenously revealed in all cases focal pulmonary congestion. The kidneys of mouse H5 showed pinhead size white nodules, surrounded by a narrow hyperemic zone. All of the animals injected intraperitoneally possessed, at the site of inoculation, small intra-abdominal abscesses walled off by mesenteric adhesions.

Microscopic pathology (Intravenously inoculated mice)

H1 and H2. Lungs: Within the connective tissue of both lungs are numerous small nodules, many parabronchial, and the others within alveolar septa (fig. 1). In the latter cases, the nodules encroach upon the alveoli, compressing them and simulating an intra-alveolar lesion. Within the centers of these bodies are numerous round, sharply defined, thick walled, brown, transparent structures (figs. 2 and 3). Some are elongated and form septate bodies which permeate the nodules. Both are characteristic of the Hormodendrum pedrosoi. About them are collected a large number of pale eosinophilic cells containing vesicular nuclei (fig. 3). Within the cytoplasm of these cells, which resemble the early epithelioid cells found in miliary tuberculosis, are numerous phagocytosed Hormodendrum spores. Scattered through these structures are many blue staining irregularly formed cytoplasmic fragments, which appear to be derived from degenerating polymorphonuclear leukocytes. Circumscribing the bodies is a moderate lymphocytic infiltration. Where a number of the tubercles have arisen in proximity to each other, the intervening alveoli are filled with phagocytes and degenerated polymorphonuclear leukocytes, producing an area of focal pneumonia.

The alveolar septa throughout are moderately thickened and infiltrated by lymphocytes. The bronchioli and bronchi are lined by intact tall columnar epithelium, and no lesions of the blood vessels are demonstrable. In no case do the tubercles show vascularization or fibroblastic proliferation. Tissue stained by the Masson trichrome method reveals a complete absence of collagen in and about the tubercles.

Livers: Hepatic lesions, similar to those of the kidneys and lungs, are present (fig. 4). They are scattered about, and appear to be associated with both central venules and hepatic veins. The portal fields show a round cell exudate.

Spleens: The follicles are enlarged and the follicular centers well developed. Reticulo-endothelial cells lining the congested sinusoids are hyperplastic. No *Hormodendrum* are seen.

FIG. 1. *H1.* Tissue reaction in lung showing parabronchial and interalveolar distribution of the lesions. (51×)

Kidneys: The cortices are marked by the presence of rare small foci of tissue reaction. Within their centers are seen the same type of Hormodendrum spores described in the lungs. Surrounding them are a large number of round cells and cells possessing very large vesicular nuclei. In but one cortical focus is there evidence of definite epithelioid reaction. In this, the *Hormodendrum* bodies are seen within the lumina of proximal convoluted tubules. In no case can involvement of a glomerulus be demonstrated.

H3. Lungs: The nodules in the lungs show the same distribution as in H1 and H2. They are somewhat larger and consist of numerous giant cells of the foreign body type. An interesting phenomenon is the fusion of these cells to form large syncytial masses which show phagocytosis of *Hormodendrum* spores, polymorphonuclear leukocytes and round cells. There are more polymorphonuclear leukocytes present in these tubercles than in those of the previous animals. The *Hormodendrum* are abundant, but restricted to the cyto-

plasm of the giant cells. Nowhere are giant cells of the Langhans type visible.

Kidneys: There are a few small lesions composed of foreign body type giant cells, mononuclear macrophages, some polymorphonuclear leukocytes and round cells similar to those described in the lungs. The typical *Hormodendrum* bodies are present. Early central necrosis is seen in a large confluent nodule.

are numerous giant cells, many differentiated into the Langhans type and containing phagocytosed *Hormodendrum*. A notable feature of these lesions is the presence of micro-abscesses composed of polymorphonuclear leukocytes within individual tubercles. They are most prominent about the fungous bodies. In some instances the microscopic abscesses fuse to form larger areas of central necrosis. In a few of the tubercles there is another type

FIG. 2. *H1*. Parabronchial tubercle demonstrating spores and hyphae of *Hormodendrum pedrosoi* surrounded by epithelioid cells and an external ring of lymphocytes. (360×)

Liver: Only a few small foci of inflammation are present in the organ. They are composed of round cells and occasional epithelioid cells. No *Hormodendrum* are seen in this section.

Spleen: The changes seen are similar to those described in H1 and H2.

H4. Lungs: In this animal some of the tubercles have further increased in size. There is some fibroblastic proliferation about the periphery, delineating the tubercles from the surrounding organ parenchyma. In the center of the tubercles

of early central necrosis, similar to that encountered in tuberculosis: coagulation necrosis with few inflammatory cells. Blue nuclear debris is scattered about within these areas.

Kidneys: The same type of lesion described in the lung is present within the cortex of the organs. Here giant cells again dominate the morphologic aspect of the tubercles. Many show differentiation into the Langhans type; some are filled with *Hormodendrum* cells; others are empty. About the *Hormodendrum* there is a polymorphonuclear leu-

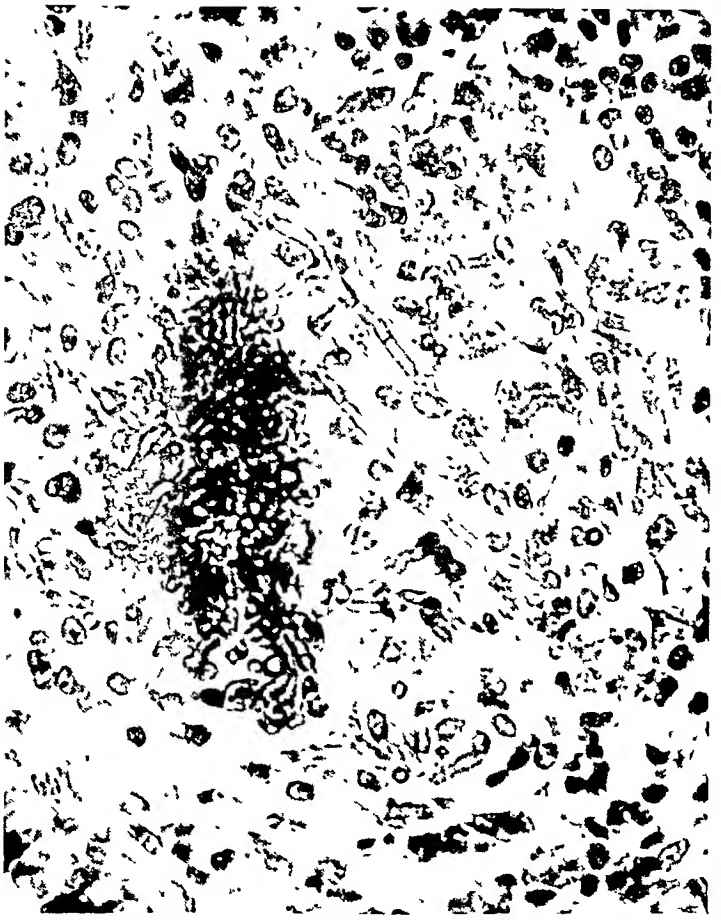

FIG. 3 *H1*. Higher magnification of figure 2. (720×)

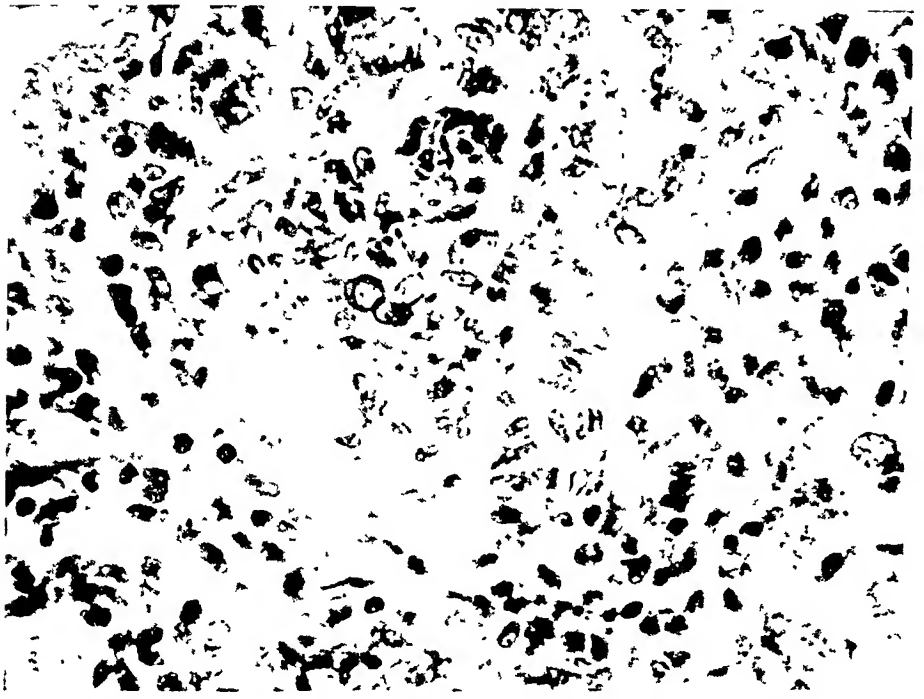

FIG. 4. *H1*. Epithelioid cell and leukocyte reaction about fungous bodies in liver. (720×)

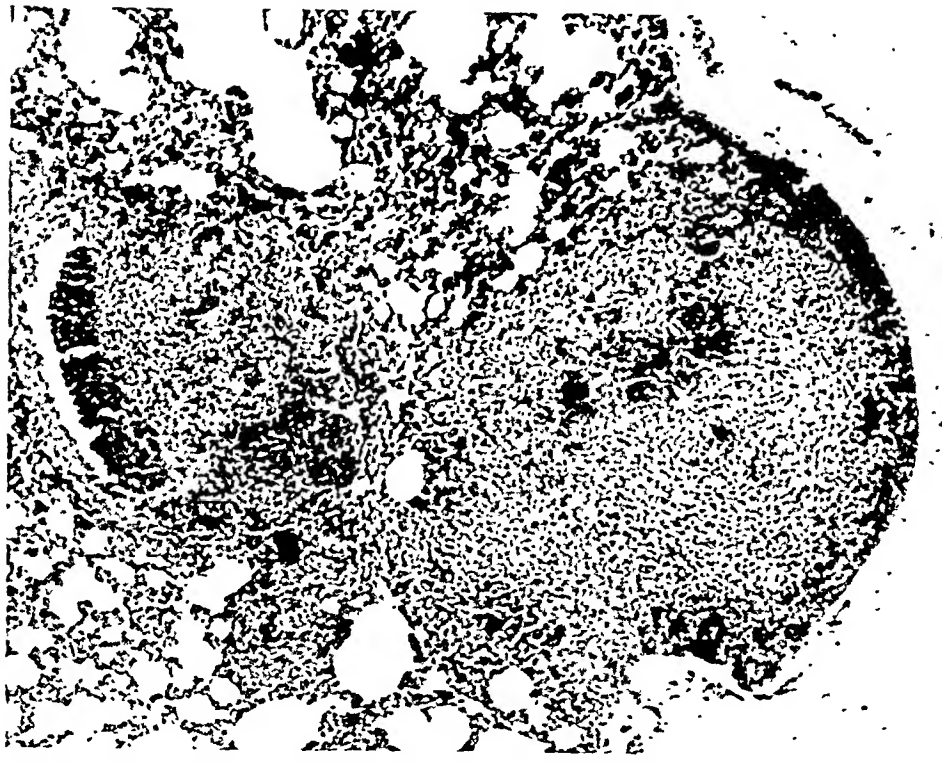

FIG. 5. *H5*. Pulmonary tubercles. The subpleural nodule contains microabscesses and shows marked fibroblastic proliferation. (108×)

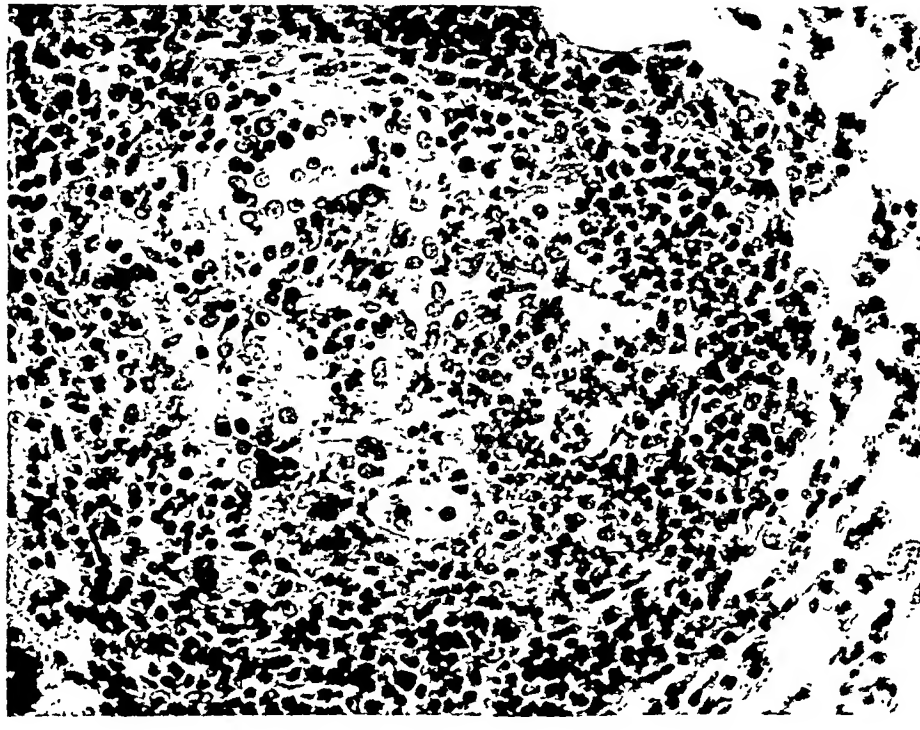

FIG. 6. *H5*. Giant-cell pulmonary tubercle. (360×)

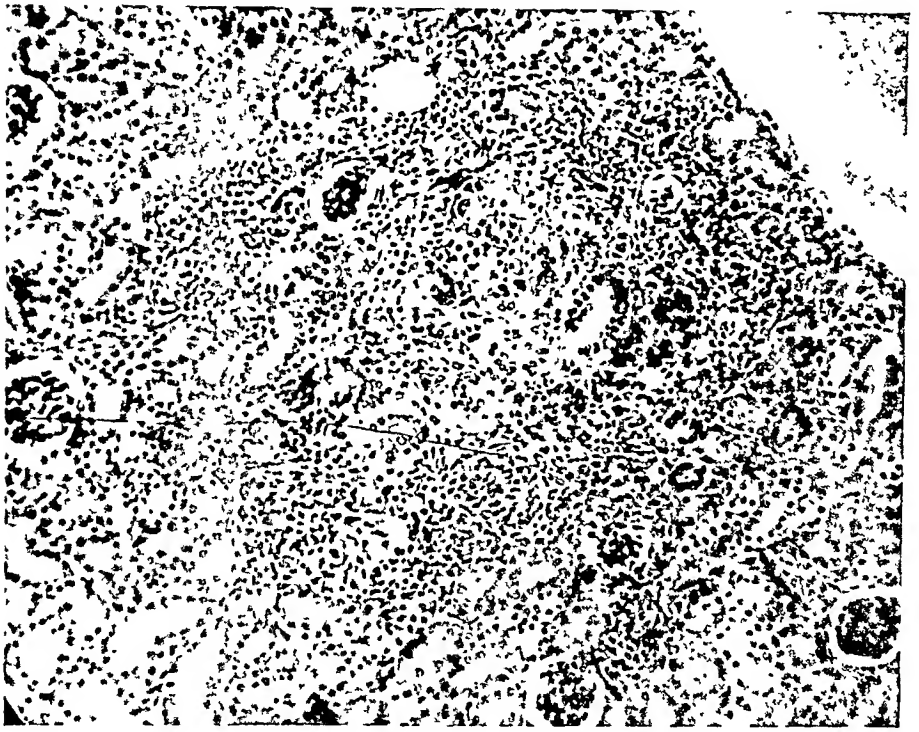

FIG. 7. *H5*. Renal tubercle characterized by Langhans giant-cells. (180×)

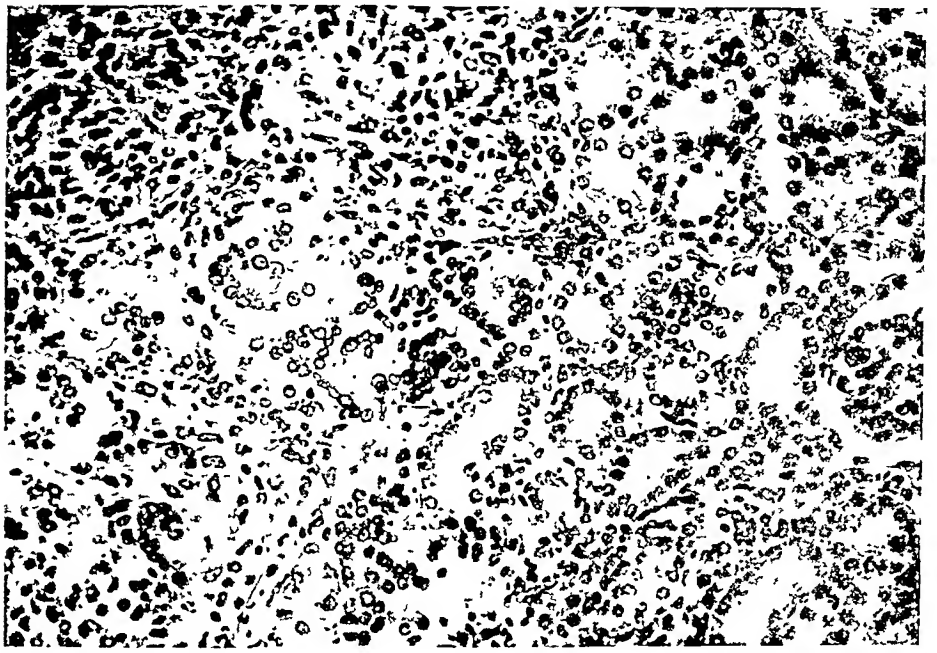

FIG. 8. *H5*. Higher magnification of figure 7 showing active phagocytosis of *Hormodendrum*. (360×)

kocytic response. Within the center of one of these tubercles early necrosis is evident. Fibroblastic proliferation is marked.

Liver: The same small focal lesions (characterized by the presence of numerous round cells, polymorphonuclear leukocytes and occasional

macrophages as described in the previous livers) are again seen.

H5. Lungs: Study of the sections reveals the process to have advanced in a few of the tubercles,

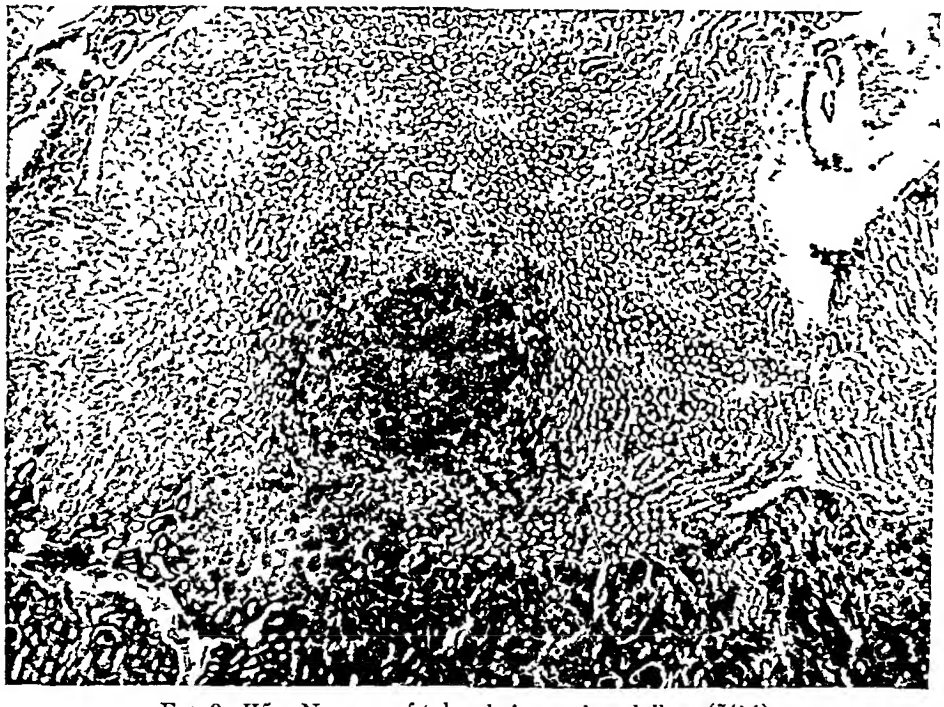

FIG. 9. *H5.* Necrosis of tubercle in renal medulla (54×)

FIG 10 *H5* Higher power view of figure 9 revealing abundance of organisms in abscess (720×)

Spleen: The spleen shows the hyperplastic reticulo-endothelial and sinusoidal congestion typical of the previously described organs.

which are now larger and marked by more definite peripheral fibrosis (fig 5). The same types of giant cells, Langhans and foreign body, both of

which show phagocytosis of the fungus, are present (fig. 6). Intra-tubercle microabscesses are prominent. There is no extension of the caseating necrosis described in the previous animal. A striking observation is the presence of small nodules, which probably represent a new generation of tubercles, since no fibrosis or scar tissue is demonstrable. They consist largely of macrophages which show ingestion of *Hormodendrum*. Masson stained tissue reveals that only the largest (oldest)

FIG. 12. *HP3.* Higher power view of abscess in figure 11. (720×)

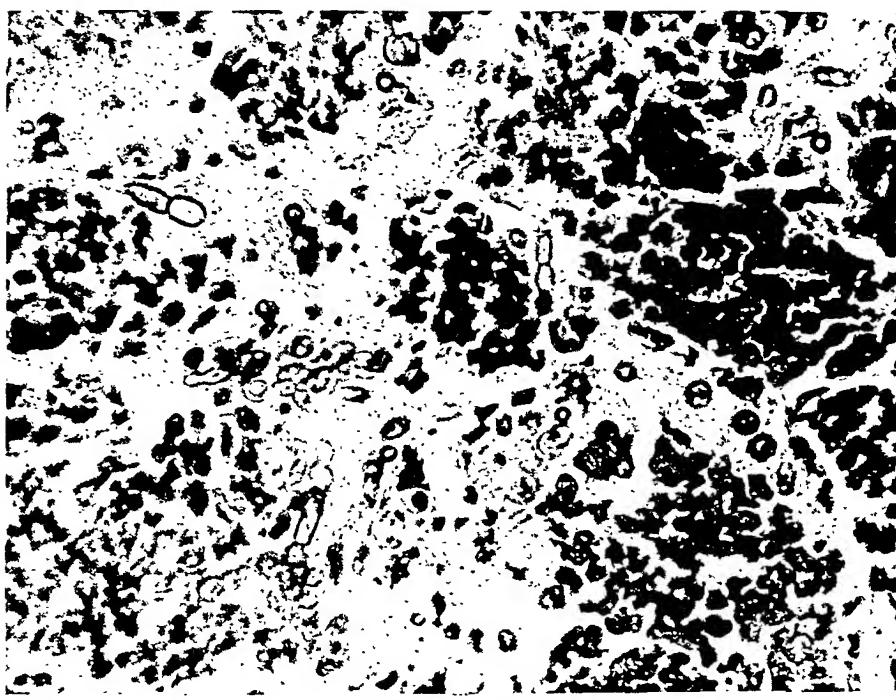

FIG. 11. *HP3.* Large nodule showing central necrosis produced by intraperitoneal injection of fungus. Stomach mucosa and muscularis intact. (36×)

tubercles are outlined by collagen producing fibres. The smaller lesions show no fibroblastic proliferation as measured by collagen production.

Kidneys: Within the cortices are lesions similar in all respects to those described in the preceding animal (figs. 7 and 8). Two large tubercles in the medulla show almost complete suppuration (fig. 9). Macrophages line the abscesses which are outlined by fibroblastic proliferation. Within the abscesses are numerous *Hormodendrum* bodies (fig. 10).

Intraperitoneally inoculated mice

HP1. The lungs, kidneys, liver, and spleen of this animal show no changes other than vascular congestion. A section through the stomach reveals intact mucosa and muscularis. The subserosa and serosa, however, are occupied by a granulomatous body measuring about one and one half mm. in diameter (fig. 11). The central portion of this nodule is necrotic and shows dense polymorphonuclear leukocytic infiltration. Encircling the whole is a wall of proliferating connective tissue. Scattered throughout the entire structure are *Hormodendrum* (fig. 12). They occur in both the spore and mycelial forms. Within the adherent omentum are smaller granulomatous structures similar to those seen in the organs of H1.

HP2, HP3, HP4, and HP5. The organs show the same lesions described in HP1.

DISCUSSION

Chromomycosis in humans is contemporaneously recognized as a disease of the skin, since no clear-cut examples of systemic spread have as yet been reported. The organism is thought to reach its human host via local cutaneous inoculation and, because of its low virulence, only a circumscribed response is elicited. These considerations influenced the design of past experiments which resulted in corroboration of the a priori assumptions.

By changing the route of the infection to the blood stream, a granulomatous lesion was produced in the lungs and kidneys of mice. The lungs, presenting the first capillary bed through which the organisms were filtered, received and apparently retained the largest number, consequently presenting the most marked lesions. The renal tubercles gave evidence of the entrance of some of the organisms into the arterial circulation. Why characteristic granulomata were not present in the liver and spleen of the older animals is not easily explained.

The formation of tubercles, both in the human skin and mouse viscera, characterized by micro-abscesses about the organisms, and the presence of spores and mycelium, is evidence of the similarity of the tissue reaction in these mammalian vertebrates.

Many of the *Hormodendrum* succumbed to the protective forces mobilized by the tissues, chiefly phagocytosis. Those which survived adjusted themselves to a parasitic existence and, as was demonstrated in H5, had already given rise to a new generation of granulomata.

The lesions produced by the intraperitoneal injection of the *Hormodendrum* were of secondary importance in this study. The basic tissue pattern elicited by this means is identical to that seen in the systemic disease. The site of inoculation is the focus of a large abscess containing numerous fungous cells and polymorphonuclear leukocytes surrounded by epithelioid cells and fibroblasts. It is evident that in HP1, HP2, and HP3 there is some lymphatic spread into the adherent mesentery resulting in the appearance of adjacent tubercles.

Three mice not included in this study were injected intravenously with *Hormodendrum pedrosoi.* All three, after 11 days, showed typical systemic lesions. The fact that 13 mice injected intravenously or intraperitoneally with *Hormodendrum* developed typical chromomycosis is evidence of the usefulness of this animal in any study directed toward the investigation of chromomycosis.

In order to study the problems of chromomycosis suggested by this work and to determine the end results of this infection, experiments embodying larger groups of animals and extending over a longer span of time have been undertaken.

CONCLUSIONS

1. Systemic chromomycosis was produced in mice by intravenous injection of *Hormodendrum pedrosoi.*

2. Focal chromomycosis was produced in mice by intraperitoneal injection of *Hormodendrum pedrosoi.*

3. The lesions elicited are similar to those described in human chromomycosis.

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OBSERVATIONS ON THE MICROFILARIA OF ONCHOCERCA VOLVULUS WITH SILVER STAINS

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In the study of the microfilaria in the histopathologic sections of the lesions of Onchocerciasis of Guatamala, one can not but be impressed with the poor and variable reaction of the microparasites to the routine tissue stains. A glance at the chapter devoted to Onchocerciasis in any of the recent text books (1–6) will serve to emphasize this. Here, the microfilaria are represented by sketches, rarely actual photomicrographs, depicting small worm like organisms of varying size and outline and a polka-dot pattern. Much emphasis is placed upon these dots—nuclear dots—column of nuclei, within the worm, inferring that the various species of microfilaria may be differentiated one from the other by the distribution and the number of the dots.

Other than these "dots" the microfilaria have few other characteristics. The remaining "cytoplasm" of the organism stains poorly, if at all, and the actual border of the organism is often not distinguishable. When the microfilaria are numerous, as they frequently are, in the center of the Onchocerca nodule they are easily found, even with the routine stains. But in the periphery of the nodule, among the dense collagenous connective tissue, their recognition as a row of dots or granules becomes difficult, uncertain and often impossible. This is especially true when one is searching for small fragments or tails.

It was obvious that a more selective stain would greatly facilitate the identification of the microfilaria and contribute to the study of their behavior in tissue, as this is as yet not well understood.

The usefulness of silver salts in the demonstration of the treponema in tissues led to the development of the following stain:

A silver stain for demonstration of Onchocerca Microfilaria in tissues

Method:

1. Fix tissue thoroughly in neutral formol.
2. Cut sections by freezing microtome, 10 microns. Note—paraffin sections can not be used.
3. Float sections in distilled water.
4. Wash sections twice in distilled water.
5. Transfer sections, (two to three at a time) to 10 cc, 2% silver nitrate solution at room temperature. Heat slowly over microburner, 60–70 degrees C., until sections become a faint but definite yellow. This may require 15 to 20 minutes.
6. Transfer sections and wash twice in distilled water.
7. Place sections in ammoniacal-silver-carbonate[1] solution for 3 to 5 min.[2]
8. Wash 1 minute in distilled water.
9. Transfer to 1% formol solution for 1 min.
10. Wash in distilled water.
11. Transfer to 5% sodium hyposulphite solution for 1 minute.
12. Wash thoroughly in distilled water.
13. Float section on slide, drain, blot with fine grade filter paper.
14. Dehydrate by flooding slide with absolute alcohol, drain, blot.
15. Clear section by flooding slide with creosote, drain, blot.
16. Mount with coverglass using clarite x.

Collagenous connective tissue is stained yellow to yellow brown. The microfilaria are colored black and stand out in contrast against a yellow environment. Nuclei and nuclear material stain black. A granular precipitate of black silver in the intercellular material indicates faulty technique. The added thickness of frozen sections aids rather than hinders the search for the microfilaria.

The suggested silver stain accomplishes two results: (1) it facilitates the demonstration of the

[1] Ammoniacal-silver-carbonate solution: (7) To 5 cc of 10% Silver Nitrate solution add 15 cc of 5% Sodium carbonate solution, mix. Add concentrated C.P. Ammonium Hydroxide Solution drop by drop until the white precipitate has been completely dissolved. Then add one or two drops in excess. Make up to 75 cc volume by adding distilled water.

[2] The length of time in silver carbonate solution will depend upon the density of the connective tissue, and upon the thickness of the section. Ammoniacal-silver-carbonate is essentially a nuclear stain, but will impregnate old collagen.

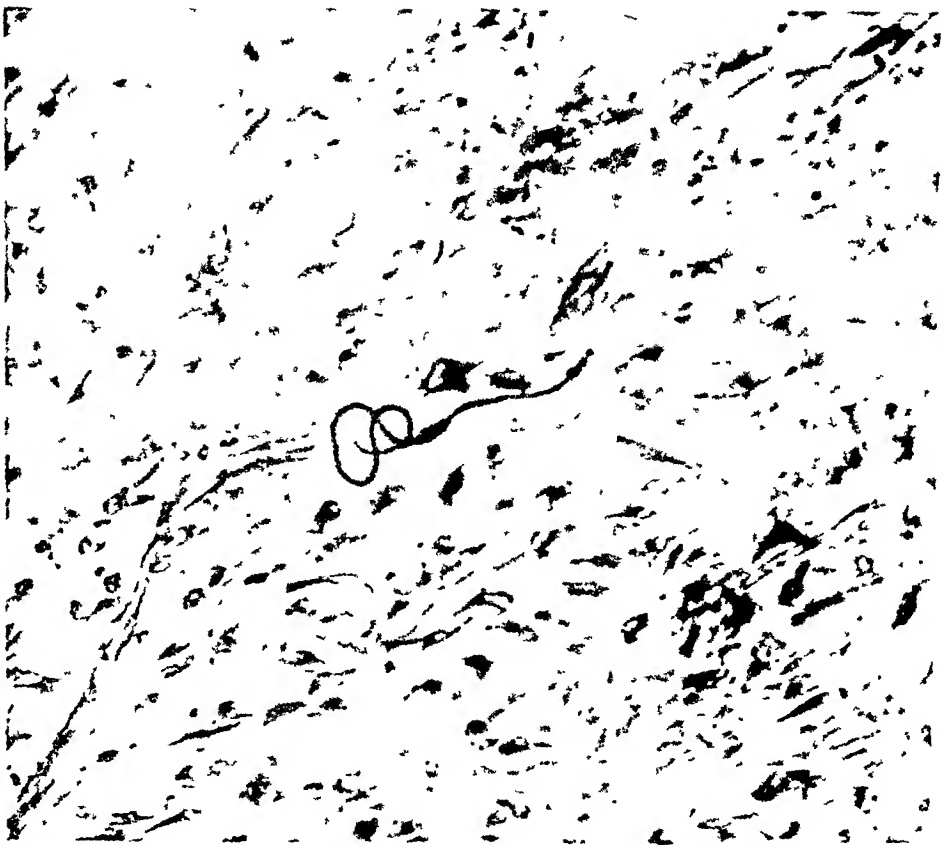

FIG 1 Microfilaria, low power in *Onchocerca* nodule. Silver stain

FIG 2 Microfilaria, high power, have striated cuticle and definite internal structure

microfilaria by contrasting them with their environment (fig. 1). This is achieved by impregnating them with silver; and (2) it visualizes a specific and complicated anatomy of the microfilaria, not described in the texts (figs. 2, 3). This added factor is of value in identifying the microfilaria when they are present in the sections in fragments (fig. 6), or in cross section.

By a combination of intensive staining and specific details shown, it is possible to demonstrate the microparasites in portions of the section such as the capsule and surrounding tissues when they are not visualized by the routine stains.

sections must be cut to find an entire microfilaria in a single flat plane. It is probable that most of the measurements of the microfilaria that have been made have been done directly upon the parasites which have been expressed from the Onchocercoma or tissues. Obviously the smaller microparasites are more easily expressed and the older and longer forms, with their marked coiling, often perivascular, cannot be removed by such a procedure and remain fast in the tissues.

FIG. 3. Microfilaria in Onchocercoma, 520 microns. Length has been underestimated

OBSERVATIONS ON THE MORPHOLOGY OF MICROFILARIA USING SILVER STAIN

Dimensions: The diameter of the microfilaria is less a variable than the length. The average estimated diameter varies from 10 to 15 microns, and is comparable to the diameter of the polymorphonuclear leukocytes seen in the same section (fig. 7, 8). The length varies from about 200 microns for the young forms in the center of the nodule to upwards of 1000 microns for older forms in outer zones of the Onchocerca nodule. Because of the tendency to extreme coiling, many

Contour-surface: The contour of the microfilaria is not smooth as has been commonly represented. It is slightly but regularly elevated by circular striations and annular condensations of the cuticle. These annular striations resemble the striations seen in adult nematodes The "striations" extend the entire length of the microfilaria from the stoma to the tip of its slender sharply pointed tail (fig. 3). Within its outer body wall can be seen some fine granules, often in short rows, beneath the striated cuticle. Striations and internal structure are not seen in the microfilaria in the uterus of the parent worm until immediately before their birth. The microfilaria during most of their intrauterine life show no

other reaction to the silver stain than the scattered granules observable by the routine stains.

Through the outer striated body wall can be seen an inner cylindrical body cavity extending the entire length of the organism to within a short distance of the tail (fig. 2, 3). This cavity is occupied largely by a column of dark and irregularly staining material which begins at the stoma (fig. 2, 3). This column is of variable diameter and consistency and quite commonly shows a slight spindle shaped enlargement a short distance from the stoma. In some places this central column is vacuolated. At some levels it may be seen as two columns which may be entwined. In a few instances three columns may be seen. These columns probably represent the precursors of the digestive and reproductive systems, though specific differentiative features are lacking. Associated with the column above described there are numerous condensations of dark staining material, usually projecting into the column. These masses are spherical, ovoid, or rod shaped and sometimes are seen in rows. These are the masses referred to in most descriptions as the "column of nuclei." They are not nuclei in the strict sense of the word, though they take the nuclear stain and may be aggregations of nuclear material.

Often the microfilaria may be seen in cross section. They present a circular appearance and the central column may be readily seen. At some levels, as suggested, the central mass appears as two, sometimes as three. With routine stains microfilaria can but rarely be identified in cross section.

The microfilaria, thus, by their structure appear as miniatures of the parent worm, which possesses a striated cuticle and an inner digestive and reproductive system.

OBSERVATIONS ON THE BEHAVIOR OF THE MICROFILARIA IN TISSUE

The number of microfilaria in the Onchocercoma is variable. When first born, they are most numerous about the center of the nodule. Here the proliferated connective tissue which has been produced in response to the presence to the parent worm is the youngest. This tissue is structurally loose and contains abundant intercellular fluid, little collagen, few fibrils. The capillaries are delicate and are but endothelial channels. In such an environment the microfilaria find locomotion easy in all planes. Extreme coiling of the parasites is observed. Their own movement takes them eventually toward the periphery.

As the periphery of the nodule is approached the density of the connective tissue increases, the amount of collagen increases and it is laid down in heavy parallel and concentric bundles (fig. 4). Microfilaria become less numerous as the density of the surrounding tissue increases. While extreme coiling of the microparasite is frequent and possible in the center of the nodule, it is customary to find the organisms in the periphery extended to conform to the parallelism of dense collagen bundles which surround them and obviously interfere with their locomotion (fig. 4). Here they are recognizable with great difficulty by the ordinary stains and while they are alive they excite no tissue reaction.

Even in the center of the Onchocercoma the microfilaria exhibit some tendency to coil about capillaries. This tendency to seek the perivascular tissue is shown in all layers of the nodule. In this area about the capillaries and small arterioles the microfilaria encounter a zone of loose connective tissue which offers less mechanical interference to their locomotion than in other areas. As the periphery of the Onchocercoma is reached the perivascular space is the site where the microfilaria are most consistently found (fig. 5). Here may be found their coiled fragments (due to plane of section), while none may be seen in the surrounding stroma.

In the perivascular space coiling is observed (fig. 6). Coiling is a manifestation of locomotion and coiling is not seen in the microfilaria that are seen between the collagen bundles remote from the blood vessels (fig. 4).

The perivascular space is obviously a path which leads to the periphery and through the capsule and therefore may be an important highway by which the microfilaria leave the nodule to migrate into more remote sites.

There are two other possible paths of exit from the Onchocerca nodule, namely the lymphatic and capillary channels themselves. Having studied large numbers of sections, in all planes, no indisputable instance was found where a microfilaria was seen within the lumen of a lymph or blood capillary. Moreover, the absence of the microfilaria from the peripheral circulation is a feature in this type of Onchocerciasis, and elephantiasis does not characterize the Guatamalan variety of this disease. This would suggest that

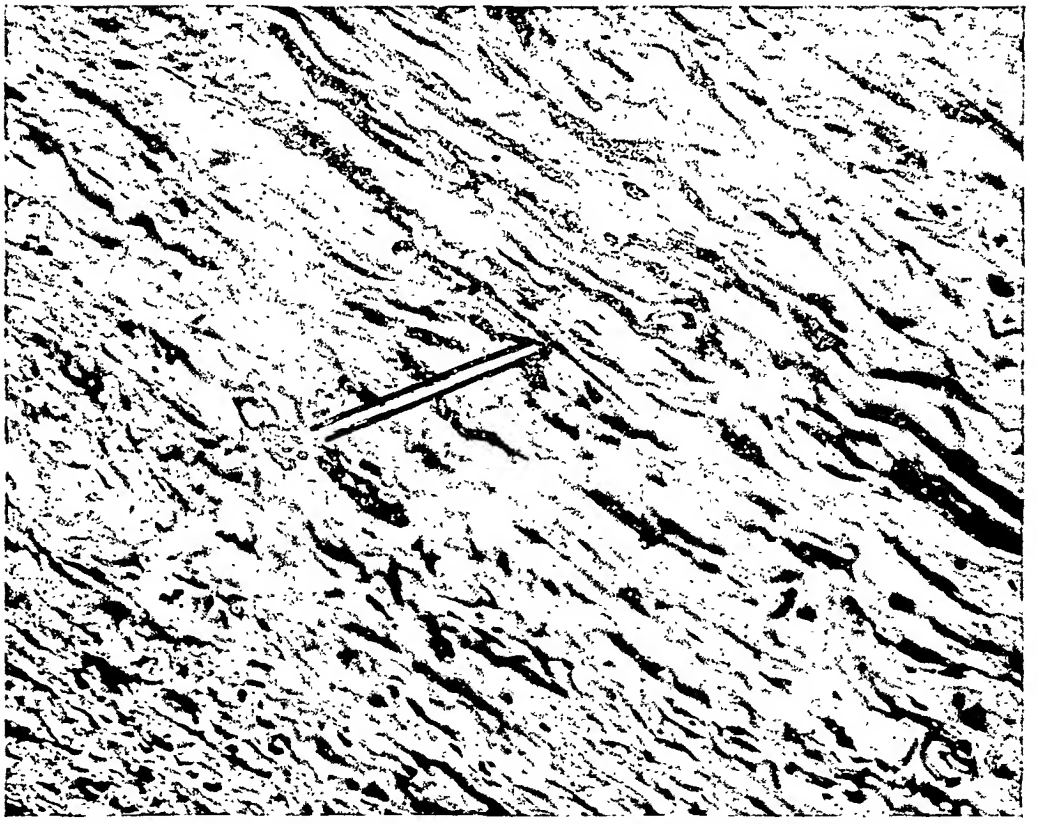

FIG. 4. Microfilaria, extended, are difficult to find between parallel bundles of connective tissue. Arrow.

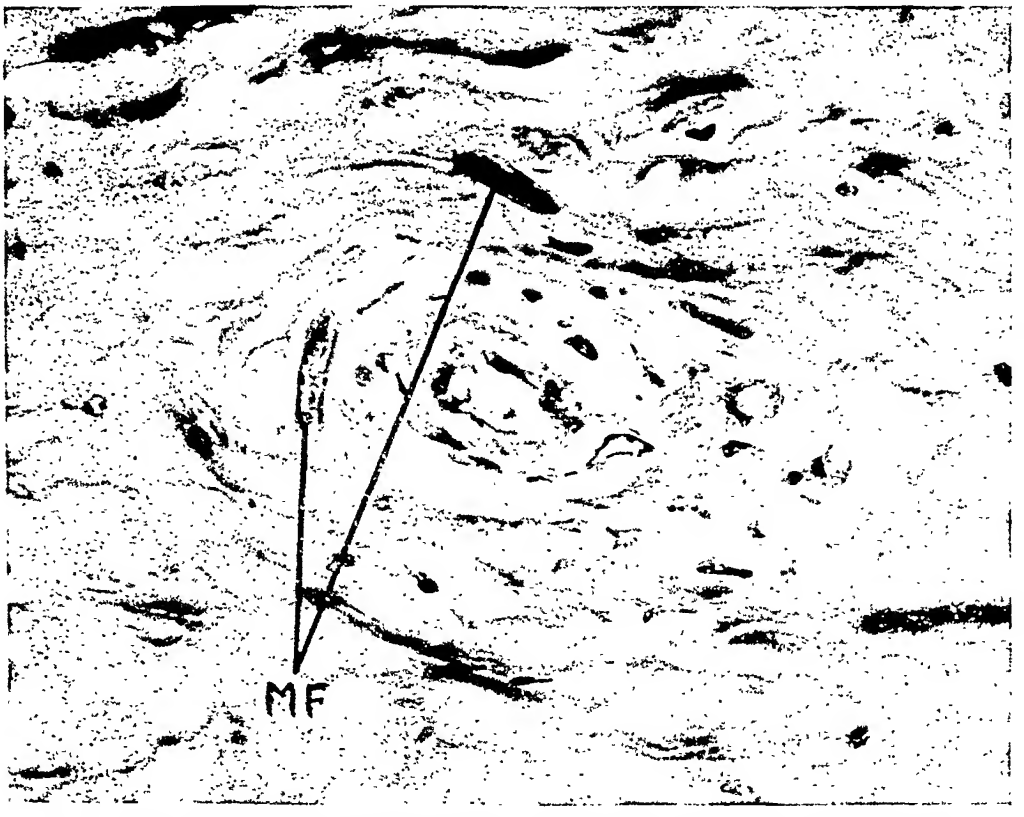

FIG. 5. Microfilaria, M. F., consistently found coiled in loose perivascular connective tissue.

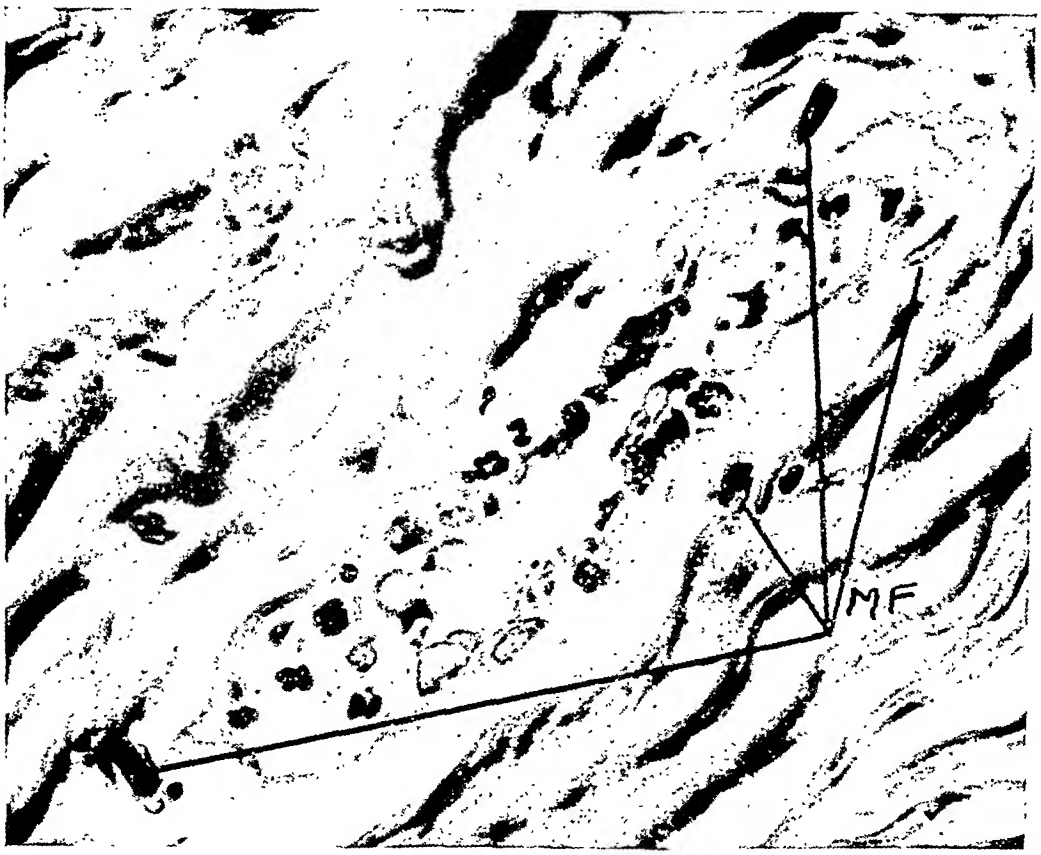

FIG. 6. Microfilaria, M. F., coiled about capillary with early capillary reaction.

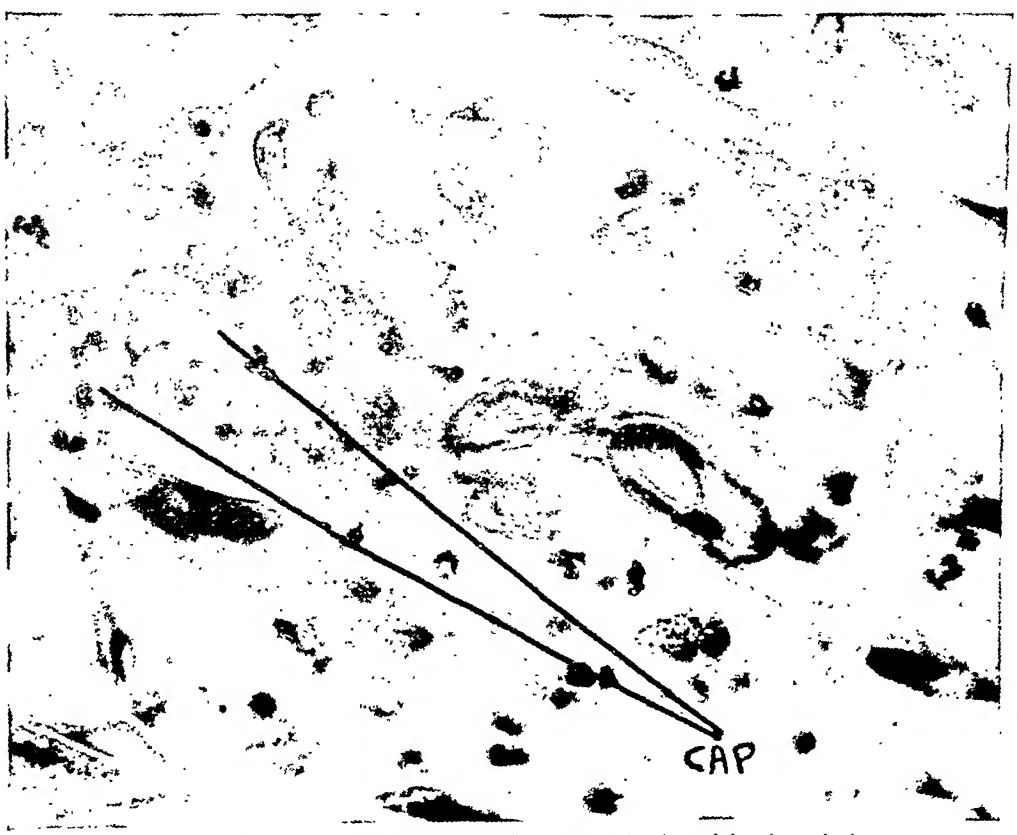

FIG. 7. Microfilaria excite capillary, CAP, thrombosis and focal exudation.

neither of these routes is used by the microfilaria as an exit from the Onchocercoma. Others have observed that the microfilaria do not migrate far from the original nodule (6). This would be consistent with the perivascular avenue suggested.

The youngest connective tissue of the Onchocercoma is in the center. Aging of tissue progresses as the periphery is reached. The capillaries in the center are those of young granulation tissue and consist of but an endothelial channel. As the central zone is left behind the capillaries acquire a connective tissue collar with collagen. In the central zone there is evidence that capillary invasion by the microfilaria is attempted. Such attempts are represented in the sections by minute focal inflammatory lesions. If one analyzes the lesions it will be seen that they are confined largely to the central thin walled capillary zone. In fact, no such lesions are found associated with capillaries in the periphery, nor in any zone where a capillary has acquired a protective wall of connective tissue with collagen.

Very frequently a microfilaria is seen in close contact with a naked capillary and the capillary is engorged with mobile inflammatory cells (fig. 6). There is an exudation of fibrin and cells into the surrounding area, with granular debris and thrombosis of the capillary (fig. 7). This indicates capillary injury with reaction. It is not unreasonable to assume that the injury was produced by the microfilaria (capillary endothelium is susceptible to injury and is reactive) and that the resultant reaction has sufficed to thwart the actual penetration of the microfilaria into the capillary lumen.

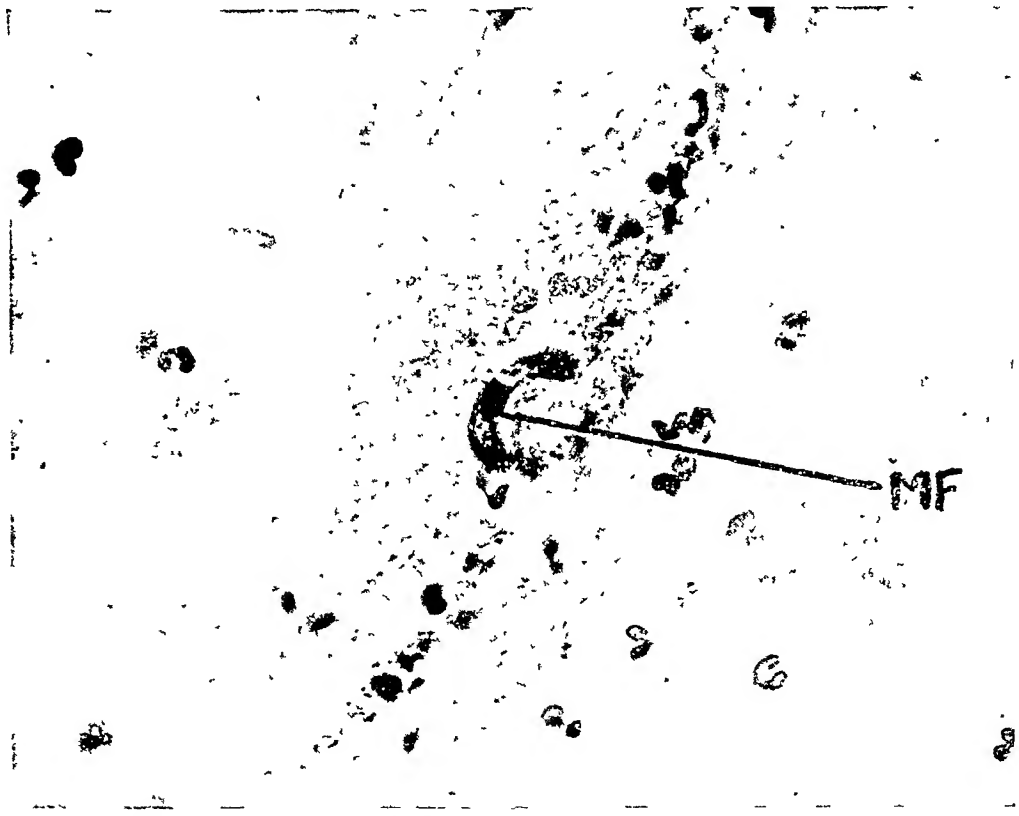

FIG 8. Capillary thrombosis, focal exudation, destruction of microfilaria, M. F.

In many instances the microfilaria are surrounded by this focal acute reaction. Among the reacting cells there are macrophages. In not a few instances the microfilaria were observed in various stages of disintegration. These stages vary from loss of visible striations to aggregation of granules into masses and loss of outline as a parasite (fig. 8).

There was no evidence that the microfilaria, either alive or dead, induced a true granuloma. While the parent parasites are alive, no giant cells were observed. When the adult parasite dies, on the other hand, granuloma formation with

giant cells becomes a very conspicuous feature and, at the same time, alteration of structure in the macroparasite is very obvious.

SUMMARY

A silver stain is described which facilitates the demonstration of microfilaria in tissues. In addition, the stain reveals details not shown by routine procedures.

Additional study would suggest that the microfilaria leave the Onchocerca nodule by the perivascular tissue spaces and not through the capillaries themselves. Evidence is present to suggest that invasion of capillaries is blocked by prompt reaction and thrombosis and that the microfilaria may be destroyed in the ensuing reaction.

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STUDIES ON THE MICROFILARIAL PERIODICITY OF LITOMOSOIDES CARINII, FILARIID PARASITE OF THE COTTON RAT[1]

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Litomosoides carinii (Travassos, 1919), a filariid parasite of the thoracic cavity of the cotton rat, *Sigmodon hispidus*, and various other rodents, has recently assumed some importance as a possible experimental animal in chemotherapeutic studies on filariasis. A search of the literature reveals that little work has been done on this nematode beyond several descriptions of the adult species, so that in conjunction with experiments on the effects of various chemotherapeutic agents on the microfilarial counts, it was thought that an estimation of the normal variation in the microfilarial counts, especially with regard to any evidence of periodicity, might be of value.

THE PARASITE, LITOMOSOIDES CARINII

In 1919 Travassos described a filariid worm from the pleural and peritoneal cavities of the squirrel, *Sciurus* sp., from Sao Paulo, Brazil and named it *Filaria carinii* (Vaz, 1934). According to Vaz (1934) this nematode is identical with *Filaria patersoni* described by Mazza in 1928 from the peritoneal cavity of the rodent, *Holochilus vulpinus*, with *Litomosoides sigmodontis*, which Chandler (1931) described from the thoracic cavity of the cotton rat, *Sigmodon hispidus* in Houston, Texas, and with *Micropleura sigmodoni* described in 1932 by Ochoterena and Caballero from the pericardial and pleural cavities of *Sigmodon hispidus* in Michoacan and Jalisco, Mexico. Vogel and Gabaldon (1932) found the worm in the pericardial and pleural cavities of *Mus decumanus* in Caracas, Venezuela and they established a new genus *Vestibulosetaria*, type species *V. patersoni* (Mazza, 1928), but according to Chitwood (1933), the correct generic name should be *Litomosoides*. Vaz (1934) found the worm in the pleural and pericardial cavities of the rodent, *Necromys squamipes*, in Sao Paulo, Brazil, and summing up all the data on this parasite, he came to the conclusion that the correct name is *Litomosoides carinii* (Travassos, 1919).

At the autopsy of rat No. 1 in our studies seven adult worms were found in the pleural cavity, four females and three males and a thorough search of the other body cavities and organs failed to reveal further worms. These conformed morphologically to the description of Chandler (1931) with the exception that the females were 115–130 mm. in length, rather than 50–65 mm. as described; most of the adult females found during the course of this experiment were over 100 mm. long. The males measured approximately 25 mm. in length.

A search of the literature reveals a brief description of the microfilariae of *L. carinii* by Vogel and Gabaldon (1932), but since some confusion apparently exists as to whether or not they are sheathed, an effort was made to demonstrate the sheaths. In his description of the adult worms, Chandler (1931) states that the embryos in the uterus of the female were 100–105 micra in length and unsheathed. Harwood (1932) refers to the parasites as sheathed in his investigation of the tissue-penetrating abilities of these microfilariae, and Vogel and Gabaldon (1932) mention the sheath and the fact that nuclei extended to the tip of the tail.

For our study of the microfilariae, thin smears of tail blood from the cotton rats were made, the erythrocytes lysed, and the slide stained with standard alum hematoxylin. The sheath was seen to be about 100 micra long (fig. 1). The cuticula was plainly visible within the sheath, especially at the anterior tip of the worm, which was usually marked by a clear area free of nuclear material extending 3–5 micra posteriorly; the deeply-stained nuclei were observed to extend to the end of the tail in most cases. With the staining technique used, the nuclei appeared to be more compact than in *Wuchereria bancrofti*.

[1] This study was made possible through financial support of the John and Mary R. Markle Foundation. During the preparation of this material, the assistance of Roger W. Williams, and Miss Bettie Ferrell, has been invaluable and to them the authors express their sincere appreciation.

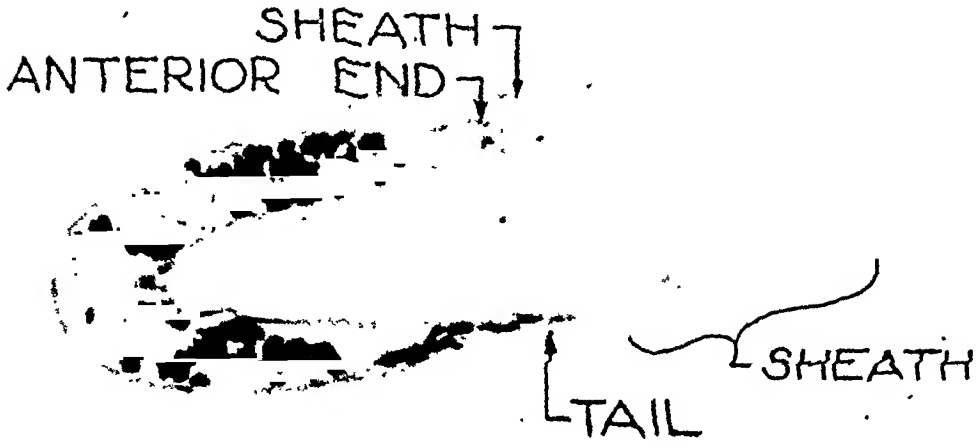

FIG. 1. Photomicrograph of *Litomosoides carinii* microfilaria, showing sheath and nuclear structure in the tail.

TABLE 1

Microfilaria (L. carinii) counts at four-hour intervals in six cotton rats over 48-hour period. The counts represent the total number of microfilariae in 3.5 c.mm. of tail blood

RAT NO.	TIME											
	6 p.m.	10 p.m.	2 a.m.	6 a.m.	10 a.m.	2 p.m.	6 p.m.	10 p.m.	2 a.m.	6 a.m.	10 a.m.	2 p.m.
1	528	397	601	574	511	615	485	539	605	500	319	516
2	231	240	345	180	100	175	275	225	150	185	280	390
3	37	10	45	30	20	55	40	40	45	75	35	10
4	300	290	315	265	225	150	240	190	370	185	235	270
5	560	445	320	415	710	675	535	455	495	455	865	375
6	130	175	135	120	130	85	105	150	120	125	85	55
Total .	1786	1557	1761	1584	1696	1755	1680	1599	1785	1525	1819	1616

METHODS OF STUDYING MICROFILARIAL PERIODICITY

The cotton rats used in this experiment were obtained from the Zoological Research Supply House in Englewood, Florida, now the Hegener Research Company, Box 224, Indian Beach, Sarasota, Florida, and were captured in that immediate region. Blood was drawn from six rats at four-hour intervals, ten, two and six over a 48-hour period by clipping the rats' tails. After the method of Brown and Williams (1945) microfilarial counts were made by drawing 3.5 c.mm. of blood into a pipette and a thick smear made to cover a measured area of 0.9 square cm. This was stained with Giemsa and the organisms counted under high power. At first the microfilariae on the entire square were counted, but Brown and Williams have showed that counting every fifth row and multiplying by five was sufficiently accurate statistically, so this method was adopted.

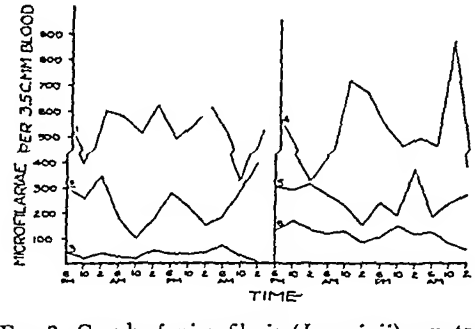

FIG. 2. Graph of microfilaria (*L. carinii*) counts from tail blood of six rats over 48-hour period.

EXPERIMENTS

Table 1 shows the results of the microfilarial counts in tail blood drawn at four-hour intervals over a period of 48 hours. Figure 2 charts the data from table 1 and demonstrates that there is no obvious nocturnal or diurnal periodicity of the parasite resembles *Acanthocheilonema perstans* which inhabits the serous cavities of humans. This random variation in microfilarial count makes the filaria of the cotton rat a difficult animal to use experimentally in the evaluation of the effects of chemotherapy in filariasis, since one cannot determine whether the counts are type shown by various human microfilariae, although the blood of each rat shows relatively wide variations in the number of microfilariae at different times.

TABLE 2

Microfilaria (L. carinii) counts grouped into day and night 12-hour periods over 48 hours

	RAT NO.						TOTAL
	1	2	3	4	5	6	
6 p.m. + 10 p.m. + 2 a.m.	1526	816	92	905	1325	440	5104
6 a.m. + 10 a.m. + 2 p.m.	1700	455	105	640	1800	335	5035
6 p.m. + 10 p.m. + 2 a.m.	1629	650	125	800	1485	375	5064
6 a.m. + 10 a.m. + 2 p.m.	1335	855	120	690	1695	265	4960

In order to check statistically whether or not the microfilariae of *L. carinii* were periodic, it was found necessary to group the counts into night, 6 p.m., 10 p.m., and 2 a.m., and day, 6 a.m., 10 a.m., and 2 p.m., counts, as shown in table 2. The data thus obtained were analyzed by Dr. John W. Fertig, Professor of Biostatistics of the DeLamar Institute of Public Health, Columbia University, by the chi-square method and it was shown that whereas with one exception each rat showed a statistically significant individual variation from one twelve-hour period to another, when the same method was applied to the total for all six rats, the variation was not greater than that which can be accounted for by chance alone.

When the above data were analyzed to show that there was a significant individual variation, there remained the possibility that there might be some sort of cyclical variation over a period of days. Consequently rat No. 1 was followed at 12-hour intervals for a total of nine days and at 24-hour intervals for a total of 20 days (table 3 and fig. 3). Although microfilarial counts as low as 223 and as high as 875 were encountered during this period, no regular cyclical variation in the counts occurred.

It is evident from the above data that microfilarial counts on the peripheral blood of rats infected with *L. carinii* will vary widely at different times and that there is apparently no way of predicting these variations. In this respect the

TABLE 3

Microfilaria (L. carinii) counts per 3.5 c.mm. of blood on Rat No. 1 at 12-hour intervals for nine days and at 24-hour intervals for 20 days

DAY	TIME	MICROFILARIAL COUNT	DAY	MICROFILARIAL COUNT
1	10 a.m.	531	9	515
	10 p.m.	397		
2	10 a.m.	511	10	845
	10 p.m.	539		
3	10 a.m.	319	11	385
	10 p.m.	223		
4	10 a.m.	266	12	545
	10 p.m.	265		
5	10 a.m.	325	13	480
	10 p.m.	455		
6	10 a.m.	375	14	485
	10 p.m.	450		
7	10 a.m.	284	15	570
	10 p.m.	620		
8	10 a.m.	862	16	875
	10 p.m.	470		
			17	825
			18	795
			19	385
			20	535

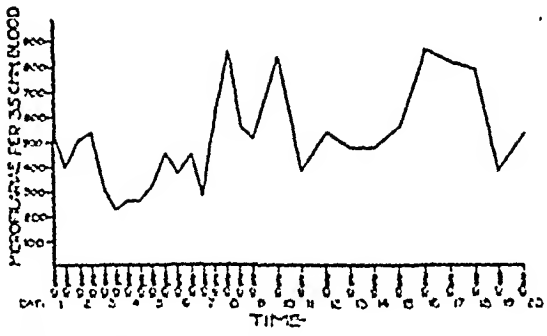

FIG. 3. Graph of microfilaria (*L. carinii*) counts in rat No. 1, taken every 12 hours for 9 days and every 24 hours for 20 days.

rising or falling spontaneously or in response to the drug. On the other hand since tests of new chemotherapeutic agents on the cotton rat filaria are based on the ability of the drug to completely rid the blood of parasites, and also kill the adults, these fluctuations in microfilarial counts, although a hindrance, do not prevent *Sigmodon hispidus* from being a good test animal.

SUMMARY

1. The microfilaria of *Litomosoides carinii*, parasite of the pleural cavity of cotton rats, does not exhibit a nocturnal or diurnal periodicity. Likewise no longer periodic cycle was encountered.

2. Variations in the microfilarial counts greater than can be accounted for by experimental error and chance alone may occasionally be encountered and may be due to variation in parturition rate, great numbers of the microfilariae invading the blood stream at one time or the death of an unusual number over a short period.

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EXPERIMENTAL STUDIES AND CRITICAL CONSIDERATIONS REGARDING THE LIFE CYCLE OF TRYPANOSOMA CRUZI

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In the life cycle of *Trypanosoma cruzi*, the causative agent of Chagas' disease, as described in most publications, the organism after an incubation period enters the blood-stream of the vertebrate host as a narrow, active "metacyclic" trypanosome, that develops without multiplication into a broad "blood form." When deposited in the tissues, it becomes a round leishmanian type that undergoes multiplication and eventually produces cyst-like accumulations. Some of the leishmanian forms may revert to trypanosomes and reinfect the blood, thus producing new metastatic foci. Since it is difficult to follow the transformation process within mammalian tissues, the developmental stages have been studied chiefly in artificial cultures and in the invertebrate host, *Triatoma*, where multiplication also begins with the leishmanian form. According to the accepted belief the life cycle of *T. cruzi* in the vertebrate and invertebrate hosts and in cultures takes the following identical course with no sexual process involved. The leishmania develop into leptomonad and crithidial forms and the latter, when mature, are transformed to trypanosomes by the migration of the kinetoplast from its prenuclear position to the posterior pole of the parasitic cell.

The experimental studies of the writer, however (1 a–e) have led to conclusions that in many respects are not compatible with the above theory. A brief description of the pertinent findings is presented. (For technics see references 1 a, b and e.)

1. Systematic examinations of the blood of infected animals during the incubational period were made in order to establish *the type of trypanosomes that first appeared in the blood.*

The popular assumption that "metacyclic" trypanosomes appear first in the blood and later develop into the typical "blood form" is not supported by direct observations. On the contrary, in the vertebrate host the blood form not only exists from the beginning, but also numerically exceeds the metacyclic form at all stages, thus making improbable the transition of the latter into the blood form. Furthermore, metacyclic trypanosomes have been followed under the microscope until all movement ceased and no transformation into the broad blood form has been observed.

Morphological differences in the trypanosomal forms of *T. cruzi* were described in 1909 by Chagas (2), who, without convincing proof, attributed to them a sexual character. His findings are often misquoted, since it was not secondary differences in size, but fundamental differences of structure that attracted his attention. My study of artificial cultures indicates that *T. cruzi* possesses, at least potentially, three different mechanisms of trypanosomal genesis: namely, (1) a direct unrolling from the round form, without other intermediate flagellate pre-stages; (2) a schizogony-like multiple segmentation process; and (3) a longitudinal binary fission. In the vertebrate, however, the first of these mechanisms seems to predominate, and the production of other types of development is still debatable.

2. The *migration theory*, which assumes that the trypanosomal form develops from the crithidial by the migration of the kinetoplast, is not based on direct observation *in vivo*, but is derived from the study of stained smears containing sufficient successive intermediate stages of kinetoplastic position to make probable the transformation of crithidia to trypanosome. Fantham (3) describes a crithidial stage in the trypanosomal development of *T. gambiense*, but the process is quite different from that of the migration theory for *T. cruzi* (1 d). These assumed intermediate stages, however, are incomplete and probably represent either the rotation of the nuclei or a retrogressive change in the trypanosomal forms. Furthermore, the intermediate stages are completely lacking in many cultures that contain abundant typical crithidias and trypanosomes. Moreover, as Brumpt (4) has demonstrated in his presentation of the life cycle of *T. cruzi*, the transformation of the crithidia into the trypan-

osome is accompanied by a considerable reduction in the size of the parasitic cell, while the development into the crithidia is associated with an increase in size. Finally, the migration theory is contradicted by the fact that the crithidia has the appearance of a mature differentiated cell, while the trypanosome resembles a juvenile cell, with structural elements quite different from those of the crithidia.

My observations on *T. cruzi* in artificial cultures (1 a) have shown convincingly that the trypanosomes develop directly by unrolling from round forms, which in turn are derived from the schizogony-like multiple segmentation of the nuclei of large cells, mostly of crithidial origin. The round forms are either the size of a rolled-up trypanosome, considerably more voluminous than the common leishmania, with the structural characteristics of the trypanosome "leishmanias of second order" or are very small forms, not much larger than a diplococcus, consisting chiefly of a nucleus and kinetoplast, with little visible cytoplasm ("elementary pairs of nuclei").

Demonstration of the evolutionary mechanism under conditions of growth in the invertebrate host, the vertebrate host, and in cultures has been possible. (a) In *Triatoma*, the writer has been able to show the existence of "leishmanias of second order" and to deduce their unrolling from the intermediate stage to the trypanosomes. Likewise, minute round forms of the "elementary nuclei pairs" have been found, and, often in a single cell group, all transitional stages up to the mature trypanosome are present. (b) In the vertebrate host Mayer and da Rocha-Lima (5) in 1914 demonstrated round forms morphologically identical with the "leishmanias of second order" and concluded that these forms unrolled directly to trypanosomes. Likewise, the "elementary nuclei pairs" and the intermediate stages of their development into trypanosomes have been found by the writer and also by Mayer and da Rocha-Lima, judging from their illustrations. (c) In cultures trypanosomal formation occurs chiefly in the blood- or cell-sediments, inside lumps of parasites and in the upper-most layers of the agar, where numerous pre-trypanosomal stages from "elementary nuclei pairs" to fully-formed young trypanosomes may be detected. From these hiding places the trypanosomes enter the supernatant fluid of the culture. At present it is difficult to determine the origin of the pre-trypanosomal stages. In cultures multiple segmentation occurs in both the crithidial and leishmanian stages and at times "microleishmania" are observed among the round forms. These minute forms are considered identical, from a morphological and evolutionary point of view, with the "small leishmania forms" in vertebrates regarded by Mayer and da Rocha-Lima (5) as an intermediate stage between the "large leishmania" and the trypanosome.

These observations indicate that the trypanosome is not, as ordinarily considered, a simple continuation of the evolution from the leishmania to the crithidia. Trypanosomes develop from the crithidia only if the latter has previously undergone a ripening process, generally combined with a multiple segmentation of the nuclei. However, this is by no means the only or most frequent mechanism in the evolution of the trypanosome form. Processes, as yet not fully explained, lead to the formation of "microleishmania" and "elementary nuclei pairs" which in turn develop directly into the trypanosome.

3. Direct observation has revealed little concerning the life cycle of *T. cruzi* in the *human body*. In the tissues obtained from biopsies and autopsies, only leishmanian forms have been found. The material from infected laboratory animals, probably due to technical difficulties, is likewise inadequate. As a rule susceptible animals are infected with strains of high virulence and when they die after a few days, their tissues are so flooded with parasites as to duplicate conditions in artificial cultures rather than those in natural infections. Nevertheless, the notable difference between the parasite in the vertebrate host and in both cultures and the invertebrate host is the almost complete absence of crithidial forms in vertebrates. It is generally accepted that the parasites are found in nests composed of leishmania or less frequently of trypanosomes, and very rarely of both, although mixed nests are more numerous than ordinarily believed. If the crithidia is a necessary link between leishmania and trypanosome and a direct prestage of the latter, it should be found at least in the mixed nests. Its absence can best be explained by the supposition that crithidias are not formed in the vertebrate, a conclusion which the writer has been forced to accept from a study of the literature and from experiments with infected vertebrates. The few vague and general references to crithidia in the vertebrates in the literature (4, fig. 148, 5, 6) are evidently the result of mistaking the

crithidia-like retrogressive stages of trypanosomes for crithidia. It appears then that the life cycle of *T. cruzi* in the vertebrate differs from that in culture and in *Triatoma* and that, in the vertebrate at least, the trypanosome form is not derived from the crithidia.

4. The retrogressive process, i.e., the gradual transformation of the trypanosome into the round or oval form which precedes multiplication, has not been studied systematically, although detailed knowledge of this process would be of undoubted importance in the correct interpretation of the parasite's life cycle. Retrogression occurs also in other stages of evolution and leads, as a rule, to the minute round form which possibly represents a resting stage.

5. The "elementary nuclei pairs" seem similar to the "latent bodies" observed in other trypanosomes by Moore and Breinl (7), Fantham (3) and Regendanz and Hoeppli (8). This fact, as well as the binary and multiple segmentations, which the writer has observed in the trypanosomal forms of *T. cruzi*, point clearly to the common origin of these species.

6. It is doubtful whether the usual practice of rejecting completely the existence of sexual processes in the life cycle of *T. cruzi* can withstand critical investigation. Biological considerations, observations of Adie (9) and Muniz (10) and my own investigations (1 a and 1 b) support the original idea of Chagas (2) that sexual processes do occur.

It is evident that our present conceptions of Chagas' disease require drastic revision, especially as they concern the life cycle of the parasite and the contamination theory of transmission (1 d), and that further extensive investigations should be conducted.

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FACTORS INFLUENCING THE UNEVEN DISTRIBUTION OF AEDES AEGYPTI IN TEXAS CITIES[1]

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Aedes aegypti is primarily a tropical mosquito. Its original home was probably Central Africa, where the Stegomyia group to which it belongs is found in greatest abundance. Being domestic in habits and finding suitable breeding places on sailing ships in the early days, it has been transported to all parts of the world. As might be expected, it has permanently established itself throughout the tropics, but has failed to do so in localities where the summers are not persistently hot, and particularly where there are long cold winters. It has become permanently established in the United States only in cities with a relatively mild winter, along the Gulf and Atlantic coasts of the Southern States.

From these perennial foci it annually extends its range inland and northward, but to variable extents in different years. In former days when good breeding places were provided on ships, it frequently became established in such northern seaboard cities as Philadelphia, New York and Boston, where it was once the cause of extensive outbreaks of dengue and yellow fever. Under modern conditions the establishment of temporary foci in distant ports by ship has almost entirely ceased. Other forms of transportation, such as automobiles and freight cars, carry only a few of the adults. This has not proved to be adequate to establish the species in northern cities in sufficient numbers early enough in the summer season to produce a large incidence before the coming of frost.

Even in local areas along the Gulf Coast and in inland parts of Gulf Coast states, the prevalence of *Aedes aegypti* varies greatly from place to place, and from one year to another. The inauguration of an *Aedes aegypti* control program in Texas in the summer of 1942 by the Office of Malaria Control in War Areas division of the U. S. Public Health Service, in cooperation with the Texas State Board of Health, made possible a study of the distribution and ecology of *Aedes aegypti* in Texas. This study has provided some interesting data, both on the actual distribution and on the factors that influence it.

A survey of Texas coast cities in the early summer of 1942 showed a very irregular and "spotty" distribution of *Aedes aegypti*. It was found to be breeding abundantly in Brownsville, Corpus Christi, Beeville, Victoria, Houston and Galveston. In most of these places the index (i.e., percentage of premises with one or more breeding places) ran as high as 30 to 40 per cent in the untidy parts of the cities. On the other hand, no *Aedes aegypti* were found in Kingsville, Freeport, Texas City, Baytown, Goose Creek, Port Arthur or Orange, in spite of the fact that suitable breeding places were abundant and *Culex quinquefasciatus* larvae, which are often associated with *Aedes aegypti*, were found in these cities. At Beaumont a survey of eight or ten blocks containing abundant potential breeding places revealed no *Aedes aegypti*, although a considerable number were found breeding in barrels for fire protection on wharves extending out over the river.

At that time no surveys were made of inland Texas cities except San Antonio, since previous observations had shown that *Aedes aegypti* was not abundant in these cities until late in the summer. In 1942 the incidence in San Antonio was lower than in the coastal cities, but in 1923, when yellow fever threatened to enter from Mexico and some control work was done in San Antonio, this species was widely distributed and abundant. In 1943 also, the index was high, running up to 20 per cent in some parts of the city in mid-summer. San Antonio thus differs from other inland Texas cities in having abundant and widely distributed *Aedes aegypti* early in the summer.

Subsequent investigation of the environmental conditions in these various cities has thrown light on the reasons for this apparently haphazard distribution of *Aedes aegypti*. In every case,

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except in the Lower Rio Grande Valley, extensive early season breeding was found to be associated with the presence of abundant "mother foci," i.e., breeding places that persist over long periods of time. Mother foci are protected from extremes of weather so that *Aedes aegypti* larvae can survive the winters in them. These mother foci have been found to be predominantly (1) cisterns, and (2) barrels for fire protection in cotton compresses and warehouses.

In the Lower Rio Grande Valley the climate is mild enough to permit the survival of *Aedes aegypti* in numbers in the absence of any great number of mother foci. Sub-freezing weather is rare, and in some years does not occur at all. In the winter of 1943–44, for instance, the minimum outdoor temperature fell below freezing on only two occasions of two days' duration each, but on the first occasion the maximum temperature for the two days was above 65, and on the second occasion the maximum fell below 60 on only one day. Such short exposures to cold are not sufficient to destroy completely either the eggs or larvae of *Aedes aegypti*, although some of the larvae die. We have had specimens of *Aedes aegypti* larvae brought into the laboratory with their siphons solidly frozen in films of ice. These larvae survived and continued development unharmed when exposed to warmth. *Aegypti* larvae left exposed to temperatures in the 30's and lower 40's for several days, however, almost invariably succumb, apparently from drowning, since they become so paralyzed by the cold that they are unable to rise to the surface. According to unpublished observations made by Assistant Sanitarian (R) Charles J. Rohde, while working on the biology of *Aedes aegypti* in the Rio Grande Valley last winter, larval activity does not cease until the temperature gets below 40°F.

Under the climatic conditions existing in the Lower Rio Grande Valley enough eggs and larvae survive in scattered exposed containers to provide numerous foci from which adult mosquitoes emerge when warm weather comes in March, so that by early summer, if uncontrolled, they are abundant and widespread. Such mother foci as cisterns and fire barrels are unnecessary for the continued propagation of *Aedes aegypti* in such localities.

In Corpus Christi, too, the short duration of cold snaps during the winter is such as to permit a few larvae to survive in scattered exposed containers, although both fire barrels and cisterns exist in sufficient number to afford hold-over places during the winter.

In Beeville almost every house is provided with a cistern, sometimes more than one. In these large, permanent collections of water the temperature does not fall low enough during the brief periods of cold weather to destroy larvae developing in them.

In Houston abundant winter hold-over places are provided both by cisterns and by barrels for fire protection. Houston had approximately 500 underground cisterns exposed to mosquito breeding in 1942. Nearly all of them were of a bottleneck type, having relatively small surface openings 4′ to 6′ in diameter, often partially or almost completely covered, but not mosquito-proof. The water in these cisterns never becomes cold enough, even during severe northers, to kill or even inactivate *Aedes aegypti* larvae. Houston also has probably not less than 10,000 fire-protection barrels in cotton compresses and warehouses where *aegypti* breeding continues almost unchecked through the winter because the cotton bales supply a surprising amount of warmth even in open unheated sheds. The adults can find protected warm places in which they can survive during cold spells and sufficiently high temperatures at intervening times to permit them to mate, bite and oviposit.

Essentially the same situation exists in Galveston, where both cisterns and fire-protection barrels are abundant. In cotton warehouses and on wharves, there are probably 7000 to 8000 fire barrels in the city. Of cisterns, 750 have been recorded. The majority are large, overhead cypress tanks of 1000 to 4000 gallons capacity, but numerous underground cisterns have been found under houses and there are a few built in attics. The winter weather in Galveston is milder than in Houston because of its seaside location. Hence it is probable that all of these cisterns, as well as the fire-protection barrels, were potential and in most cases actual winter hold-over places of *Aedes aegypti* larvae. While many of the larvae probably develop into adults during warm periods in winter and then generally fail to survive, there is no doubt that some succeed in biting and depositing eggs. Therefore, Galveston had *Aedes aegypti* foci scattered all over the city when warm weather came in the spring.

From these foci miscellaneous secondary breeding places in rainfilled containers and water plants could be seeded with eggs. It is not surprising, therefore, that Galveston had a widespread high index of *Aedes aegypti* breeding, running up to 40 per cent in some sections of the city.

In contrast to Houston and Galveston the situation in Texas City, Baytown, and other neighboring cities is astonishing. A survey of the most likely parts of Texas City in July, 1942, failed to reveal a single breeding place of *Aedes aegypti*. Texas City is less than ten miles from Galveston and has abundant potential back-yard breeding places. It does not, however, have cisterns, and all its fire-protection barrels are located on wharves under the control of a single company. All these barrels had been treated with cresylic acid disinfectants in sufficient quantity to prevent *Aedes aegypti* breeding, although Culex had started breeding in many individual barrels. The lack of these winter hold-over place evidently prevented the survival of sufficient *Aedes aegypti* to start breeding in the spring. While it is always possible that a few adult males and females, or mated females, might be imported in automobiles or freight cars early in the season and start a focus from which the entire city could be seeded by mid-summer, this failed to occur in 1942, 1943, or 1944.

The same situation exists in the cities of Harris County outside of Houston, such as Baytown, Goose Creek and LaPorte. Spot checks of these cities failed to reveal any *Aedes aegypti* breeding places in July, 1942. In late August of 1943 a similar check revealed a single premise in Baytown in which two containers were discovered with larvae, but without pupae. It seems probable that these containers were seeded by adults imported in an automobile. Had this happened in May or June, this city might have had widespread *Aedes aegypti* breeding by late summer. Places without winter hold-over places but situated in proximity to cities like Houston or Galveston, therefore, constitute a separate problem each year. Although they may be entirely free of *Aedes aegypti* breeding in one year, this is no guarantee that they will be so in another year.

An unusual situation was found in San Antonio. In spite of the fact that this inland city has a more severe winter climate than any of the Gulf Coast cities, and has no cisterns and very few fire-protection barrels, it differs from other inland Texas cities in developing a prevalence of *Aedes aegypti* breeding in the early part of summer. The reason for this did not become apparent until it was discovered that there are a number of large areas on the outskirts of the city where there is no piped water supply. In these places drinking water is either delivered by trucks and stored in barrels or tubs or is drawn from shallow wells. Most of the temporary storage places for drinking water are located outside the houses, where cold weather destroys all of the larvae and most of the eggs during the winter. A fraction of the eggs could survive if they remained unhatched, but during periods of warm weather intervening between cold snaps, they hatch and the larvae are destroyed by subsequent cold weather before they are able to mature.

The shallow wells, on the other hand, constitute good mother foci, since they are deep enough in the ground to be protected from the extremes of cold. Active breeding, although slow, takes place in them throughout the winter. Many are lined with wood, brick, stone or concrete, but several with only hard clay walls were found to be breeding.

In the spring, adult mosquitoes emerging from these mother foci begin breeding in great numbers in stored drinking water in barrels and tubs. The hordes of mosquitoes later emerging from these find ideal hiding places in automobiles parked alongside of their breeding places, for many of the residents of this area have cars, but few have garages. The mosquitoes might then be carried into the city where, when liberated, they could start numerous new foci from which the entire city could become infested fairly early in the season.

The situation in Beaumont in July, 1942, was interesting in comparison with this. Here, as already remarked, no breeding places were found in the parts of the city where it was thought they would be most likely to occur. The only exceptions were some untreated fire-protection barrels on wharves built out over a river where bales of cotton were stored. In this case, however, no parking places for cars were located within flight range of the mosquitoes, and the prevailing breeze was out over the water. So the opportunities for the mosquitoes to be carried to other parts of the city and successfully establish themselves in scattered places were minimized. By these fortuitous circumstances Beaumont was

apparently protected from an early prevalence of the mosquitoes, even with a focus right at hand. In 1943 somewhat more extensive breeding was found in this city. Possibly some of the mosquitoes which were breeding on the wharves succeeded, later in the season of 1942, in escaping to other parts of the city, perhaps in trucks in which cotton bales had been delivered. Thus dispersed, they apparently were successful in surviving the winter of 1942–43 in more scattered places.

The available evidence with respect to the overwintering of *Aedes aegypti* on our Gulf Coast suggests that some winters may be much more favorable for survival than others, and that the distribution as well as the total duration of cold weather may be important. Rozeboom (1939) found that in Stillwater, Oklahoma, large numbers of eggs kept dry on sand throughout the winter, and when not exposed to rain or snow, produced vigorous adults. During the winter there were several periods when the temperature dropped considerably below 0°C. for four to nine days in succession. Eggs that were exposed to rain and snow, however, failed to produce any adults. Assistant Sanitarian (R) S. P. Hatchett, working with me in Houston, Texas, during the winter of 1943–44, found that a high percentage of eggs hatched after being exposed to the Houston winter, whether kept dry or immersed in water. Here, however, during the course of the experiments, the minimum daily temperature fell below freezing only four times, and only once on two successive days. The maximum temperature was above 60 on most of the days, rising to between 70 and 75 on some occasions. The result was that the majority of the wet eggs that were viable produced larvae in the course of the winter. The great majority of these larvae, when exposed to the extremes of cold, failed to survive, especially if not well fed. Larvae that had recently molted were especially susceptible. On the other hand, larger numbers of larvae survived in containers which were partly buried in the ground or in piles of loose debris, especially when there was a layer of decomposing leaves on the bottoms of the containers. Under these conditions the larvae matured very slowly, requiring up to 55 days before pupating, and only 20 per cent succeeded in reaching the adult stage.

In the light of these data it seems probable that submerged eggs exposed to alternations of cold and warm weather may produce fewer adult mosquitoes than those exposed to continual cold. A warm spell of weather will permit some of the eggs to hatch into larvae. A subsequent cold snap will then destroy a considerable portion of these. If a few survive and finally become adults before the last cold weather of the winter, these are very likely to be destroyed unless they find shelter indoors, since they die within an hour at temperatures below 40°F. The extent to which *Aedes aegypti* survives the winter along the Gulf Coast, therefore, probably depends on the manner of alternation of cold and warm periods of weather more than it does on the severity of sudden cold snaps or on the mean temperature for the winter. If it were not for the fact that the eggs hatch at very irregular intervals (from $1\frac{1}{2}$ to 43 days after immersion) the mortality would probably be still higher.

In small communities, then, there may be considerable fluctuations from one season to another in density of *Aedes aegypti*, or even in its presence or absence. This fact is of importance in connection with control programs. This fluctuation could be due to variations in the distribution of cold weather in different winter seasons, or to the chance introduction of *Aedes aegypti* from neighboring infested cities, or from more or less isolated foci. Failure to find *Aedes aegypti* in Baytown or Texas City, for instance, in one season does not necessarily guarantee that a similar condition will exist in another season. The chance introduction of males and females, or of impregnated females, from Houston or Galveston, respectively, might result in an early start of breeding in these communities in any year, resulting in a widespread prevalence of the species by mid-summer. Resurveys of such places are, therefore, necessary in different seasons and from time to time in a single season. Comparison of the incidence of breeding in Beaumont in 1942 and in 1943, referred to above, demonstrates the truth of this hypothesis.

In control operations directed towards a rapid reduction of the *Aedes aegypti* population of a city, it is obvious from the observations reported above that particular attention should be directed towards permanent or semi-permanent "mother foci" from which such secondary breeding places (such as tin cans, tires, roof gutters, animal drinking pans, containers holding water plants, etc.) are seeded. During the summer these secondary breeding places are able to maintain themselves irrespective of the larger and more permanent

foci, and a large part of a control program, both inspectional and educational, may have to be directed against them. During the winter, however, attention to the mother foci may result in a very marked delay in the prevalence of breeding in secondary breeding places in the following season, and in a gratifying reduction in *aegypti* density, at least until late in the season.

This has been fully demonstrated in Galveston. An initial survey in the summer of 1942 revealed *Aedes aegypti* breeding in as high as 40 per cent of the premises in some of the worst sections of Galveston, close to the heart of the city. In the summer of 1944 the breeding index in these sections had been reduced to from 1 to 3 per cent. While part of this reduction was unquestionably due to inspectional work and public education, we are convinced that a considerable part of it was due to the abolition of mother foci by destruction, sealing, stocking with Gambusia, mosquito-proofing or larviciding of cisterns, and the larviciding of fire barrels.

Such measures might suffice for the complete elimination of *Aedes aegypti* in relatively small communities such as Texas City or Port Arthur, but would probably never be sufficient for this purpose in cities as large as Galveston, San Antonio or Houston, since the chances of this mosquito surviving the winter in hidden mother foci such as flooded basements, sumps, etc., or even in occasional protected secondary foci, are increased more or less proportionately to the size of the city.

SUMMARY

The prevalence of *Aedes aegypti* in early summer in Texas cities is very variable. Only in the Lower Rio Grande Valley is the winter climate mild enough to allow this mosquito to survive, without protected hold-over places, in sufficient numbers to produce a high incidence of breeding early in the season every year. In other cities early prevalence of breeding is associated with abundance of protected winter hold-over places, especially cisterns, fire-protection barrels in compresses and warehouses, or shallow wells. In cities having only a few such hold-over places, a high breeding index does not develop until late summer, and in many smaller cities such an index may not develop at all unless the mosquitoes are imported from elsewhere in automobiles or freight cars. In such cities the occurrence and prevalence of *Aedes aegypti* may vary from year to year depending on whether or not such an importation occurs early in the season. In coastal cities, alternation of cold and warm periods during the winter has a harmful effect on the survival of *Aedes aegypti*, and may be more important in holding the insect in check than the extremes of cold, or the total amount of cold weather.

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IMMUNIZATION AGAINST MALARIA

Unsuccessful Attempts to Increase Resistance of Ducklings to Plasmodium Lophurae Infections by Previous Injections of Materials Containing the Forssman Antigen

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As a part of the general study of immunity in malaria, it was noted that animals known to have the Forssman antigen in their tissues largely represent the group of vertebrates that are not hosts to malaria. Upon closer examination of this coincidence, there appeared to be a high correlation between these two apparently unrelated phenomena. The pertinent data appear in table 1. The hapten, when present, usually occurs in the organs but not in the erythrocytes. In the sheep and goat, it occurs only in the erythrocytes; in the chicken, in both the organs and the erythrocytes.

TABLE 1

The animals in which the Forssman hapten is present and those in which it is absent (Boyd, 1)

The animals known to be hosts to species of malaria are indicated by an asterisk (*)

HAPTEN PRESENT	HAPTEN ABSENT
chicken*	cuckoo*
ostrich	cross bill*
turkey bustard	goose*
whale	owl*
horse	pigeon*
camel	sparrow hawk
goat	wagtail
sheep	cattle
guinea pig	deer
hamster	pig
mouse	roe
dog	rabbit
fox	rat
wolf	several monkeys*
cat	chimpanzee*
lion	gibbon
ocelot	macacus rhesus*
puma	man*
tiger	baboon*
lemur	

Plasmodia have been found in the jumping rat, the water buffalo, and the duiker antelope; whether these animals have Forssman antigen is not recorded, but they will possibly resemble their relatives in the second column. The parallelism between the absence of the hapten and the susceptibility to malaria is striking; likewise, with one exception (chicken), there is a remarkable absence of observed malaria among animals having the antigen. The one exception, the chicken, is itself unusual, in that the antigen occurs in both the organs and the erythrocytes.

Although many kinds of disorders and conditions seem to influence the susceptibility of animals to malaria, among which may be named various dietary effects, injections of materials certainly not possessing antigenic powers, and increasing age, it was thought worth the trouble to investigate the effect of prior spaced injections of tissue material known to contain the Forssman hapten.

MATERIALS AND METHODS

A strain of *Plasmodium lophurae* was used which originated from Dr. E. K. Marshall's laboratory. Pekin white ducklings from a commercial source served as the experimental animals. The materials employed as a source for the hapten were horse kidney, guinea-pig kidney, and sheep red cells. In each instance, care was taken to prepare the material for use as rapidly as possible, in order to avoid the effects of bacterial activity and autolysis. The doses were purposely large; and the injections were spaced from two to five days apart, in three to six doses subcutaneously. The measures of an increase in resistance to a standard infecting dose (10 million plasmodia intravenously) were: (1) survival past the twentieth post-infective day; (2) unusually low percentage of parasitization of the erythrocytes; (3) absence of obvious signs of illness. These indications, in combination with proof of virulence

of the infecting dose as shown by fatal infections in the control animals, were accepted as evidence that the manipulations performed upon the test animals had had some beneficial effect.

EXPERIMENTS

(1) *Guinea-Pig Kidney Suspensions*

Several experiments with this material were performed. It was found that ducklings given subcutaneous injections of one-half cc. of fresh suspension (1–10 in saline) of guinea-pig kidney in four doses separated by three days were not benefited; five test animals died on the ninth and tenth post-infective days with severe infections (90 per cent or more of the red cells parasitized). They behaved exactly like the control animals. In the same experiment, a portion of the guinea-pig kidney suspension was heated in a boiling water bath for one-half hour before injection. Of three ducklings treated with this material, two showed an increased resistance; one survived after a mild infection (3–5% parasitization of red cells), and the second died on the thirteenth day, when peripheral parasitization had declined to less than 25 per cent.

The second experiment of this series, therefore, made use of heated guinea-pig kidney suspensions. A 1–10 saline suspension was heated in the autoclave at 10 pounds pressure for 15 minutes, and one cc. doses four days apart were given three times. Nine test ducklings and three controls were used, with the result presented in table 2.

Six of nine test ducklings survived an infection which killed all of the control animals. This is an unusual result, because the strain used in this laboratory will uniformly kill 80 per cent of all untreated ducklings before the tenth post-infective day.

In order to confirm this finding, another experiment was performed along the same lines. The guinea-pig kidney suspension was prepared in the same manner, and six test ducklings were injected subcutaneously with one-half cc. doses four times, two and three days apart. However, of the six animals only one survived beyond the ninth post-infective day; all the others died of malaria on the eighth and ninth days. In the same experiment, six additional ducklings were given one-half cc. of 50 per cent hog serum mixed with the guinea-pig kidney suspension; these ducklings all succumbed as well.

Because of the failure of this second attempt, the method of preparing the guinea-pig kidney material was changed in a number of ways: (a) larger doses were used; (b) they were spaced farther apart; (c) the suspension was separated into sediment and supernate; (d) hog serum alone was used; (e) the suspension was heated in a water-bath; (f) sera from rabbits immunized against guinea-pig kidney were used therapeutically. All of these variations of method yielded only negative results; the experience of the first promising experiment could not be repeated.

TABLE 2

WEIGHT OF DUCKLINGS, 1ST POST-INFECTIVE DAY	PARASITIZATION OF ERYTHROCYTES: PERCENTAGES ON—POST-INFECTIVE DAY								
	7th	8th	9th	10th	11th	12th	14th	18th	25th
gms.									
Test animals									
664	5	5	5	3	0.5	v.r.*	0	0	0
680	10	5	v.r.	0	0	0	0	0	0
472	40	50	20	5	1	1	0	0	0
700	60	80	70	25	10	10	0	0	0
700	50	50	80D†						
736	50	80	80	25	1	0	0	0	0
568	50	90	95D						
556	70	90	90D						
610	70	70	50	50	25	15	0	0	0
Control animals									
820	60	90	90D						
630	70	90	90D						
660	70	90	90D						

* v.r. = very rare parasite seen.
† D = died from malaria.

(2) *Horse Kidney*

Horse kidney also contains the Forssman antigen. It was therefore employed as source material for experiments similar in outline to those described above. The results were as follows:

	20-day survivors
(a) Heated horse kidney suspension.....	2/7 (2 of 7 test ducks)
(b) Hot alcoholic extract	2/6 (2 of 6 test ducks)
(c) Hot alcoholic extract with hog serum...	0/7 (none of 7 test ducks)

(3) *Sheep Erythrocytes*

Sheep cells were washed with saline and then used as source for material to be injected in

similar experiments. Seven ducklings were given five subcutaneous doses of autoclaved washed sheep cells. None survived a standard infection.

(4) *Infected Duck Erythrocytes*

In a similar experiment, duck's blood heavily infected with *P. lophurae* was washed with saline, then heated in autoclave for 10 minutes at 15 pounds. No protective effect was found.

COMMENT

The experiments described in this paper appear not to support the thesis that the thermostable haptens contained in the materials used for "immunization" arouse any kind of useful defense against malarial infections in young ducklings. The result of an early promising experiment could not be brought out again, even though a number of variations in procedure were tried.

SUMMARY

There is an intriguing parallelism between the known absence of the Forssman hapten in man and certain animals and the known susceptibility of man and these animals to plasmodial infections. Experiments outlined in this paper designed to increase the resistance of ducklings to infections of *P. lophurae* by preliminary treatment with materials containing this hapten have yielded largely negative results.

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A STUDY OF THE EFFECTIVE FLIGHT RANGE, DENSITY, AND SEASONAL FLUCTUATIONS IN THE ABUNDANCE OF ANOPHELES QUADRIMACULATUS SAY IN DELAWARE[1, 2]

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The epidemiologically effective flight range of the vector is an important factor to be considered in any malaria-control program, however large or small. The entire anti-mosquito aspect of the problem can often be arbitrarily limited to a zone of 1 to 2 miles about the point or points at which it is desired to attack the disease. *Anopheles quadrimaculatus* Say, for example, has usually been considered capable of movement in significant numbers only for a distance of about 1 mile from its breeding areas. The evidence is very convincing that, under normal conditions, control of the breeding of this species for a distance of 1 mile about a locality will insure protection against malaria in that locality. This practice has been utilized by the Tennessee Valley Authority in its huge anti-malaria program. The conclusions of LePrince and Griffitts (1917), Smith et al. (1941), King et al. (1942), and others to the effect that the flight range of *A. quadrimaculatus* is not, as a rule, over 1 mile were never doubted by the writers. However, it was suspected that in an area of unusually heavy production, there might be an appreciable movement of this species beyond this accepted 1-mile distance. If such were the case, it would not always be a safe practice to limit anti-mosquito measures to a zone of 1 mile.

The conditions in the present study-locality (Fort duPont, Delaware) are such as to greatly facilitate an investigation of the flight range of this species, owing to the very high productivity of the general area and to the additional fact that very small numbers are produced outside of its central portion. The later sections of this paper will, it is believed, amply substantiate the statement that this locality is a heavy producer of *A. quadrimaculatus*.

Previous records for this species at Fort duPont had shown its very high abundance there. Within a radius of 4 miles from this Post, two large fresh-water marshes, the St. Georges Basin and the Dragon Creek Impoundment, are present, both of which are heavy producers of this species. The former includes about 1200 acres, three-fourths of which is productive, and the latter about 250 acres, all of which is productive. The vegetation in each case is naturally conducive to the development of this anopheline.

There is present a considerable diversity of dominant plants which occur in rather localized areas. These dominant species, such as *Typha angustifolia* L., *T. latifolia* L., *Sagittaria latifolia* Willd., *Pontederia cordata* L., *Nymphaea advena* Ait., and *Persicaria spp.*, are found along with numerous associated forms of such genera as *Potamogeton*, *Lemna*, *Elodea*, *Ceratophyllum*, and *Utricularia*. The hydrogen-ion and salt concentrations have generally been favorable to *A. quadrimaculatus* production in both areas. In the larger area, where the most intensive breeding of this species occurs, pH values are neutral or slightly alkaline. The smaller area, with pH values neutral to slightly acid, tends to produce *Anopheles walkeri* Theob. in greater numbers than *A. quadrimaculatus*. A view of a typical breeding situation, with principally *Sagittaria latifolia*, is shown in figure 1.

METHODS USED IN THE FLIGHT RANGE EXPERIMENT

Four cattle barns or sheds were selected at each of six distances from the nearest point of major breeding in either the Dragon Creek or St. Georges Basin areas. Each distance was, there-

[1] This paper is the result of work carried on under the joint sponsorship of the Delaware Agricultural Experiment Station and the Delaware State Highway Department, Mosquito Control Division. The study was accomplished under the supervision of Dr. Paul L. Rice, Acting Head of the Department of Entomology of the Experiment Station. This is publication 193 and scientific article 119 by that department.

[2] The authors wish to express their appreciation to Dr. L. A. Stearns for suggesting the investigation and for valuable contributions toward the plan of the work; and to Dr. Paul L. Rice, for many useful suggestions concerning the manuscript.

[3] At present, with the Health and Sanitation Division of the Institute of Inter-American Affairs, Bogota, Colombia, S. A.

fore, replicated 4 times. The distances selected were 0.1, 0.5, 1.0, 1.5, 2.0, and 3.0 miles, respectively. No means was available to control the small amount of breeding that may have occurred in local situations closer to the stations than the two major areas from which these distances were taken. Hence, no claim is made that this is a critical study of the absolute flight range of this mosquito, although it is felt that the results are fully conclusive as further verification that the effective flight range in areas of normal production is not over 1 mile. Two nail kegs were placed in sheltered positions near each barn or shed.

Generally, 17 inspections were made of each barn or shed during the summer. The numbers of mosquitoes present at each inspection were estimated, and a sample was taken for later identification. Estimates were based upon actual counts in sample areas representative of the total area in each station, and upon identifications of the mosquito samples. The combined sample areas in each station varied from one-fifteenth to one-fourth the total area, depending upon the nature of the building and the general abundance of mosquitoes, but the same sample areas in each station were used at all inspections. For the stations closest to the breeding areas, approximately 1200 mosquitoes were identified as to sex and species for each station during the period of the study. Obviously, stations with low densities did not furnish such a large sample for identification.

FIG. 1. TYPICAL A. QUADRIMACULATUS BREEDING CONDITIONS IN THE FORT DUPONT, DELAWARE AREA

VARIATIONS IN DENSITY AT VARYING DISTANCES FROM THE PRODUCTION CENTER

Table 1 includes the estimates of the numbers of this species present per inspection per cattle barn or shed. The numbers collected from the nail kegs are also shown. The data in table 1 (which are presented graphically in figure 2) clearly reveal that there is a very marked decline in the numbers of this species at increasing distances from the breeding area.

Furthermore, it is obvious that there is a close correlation between the records obtained from barns or sheds and those obtained from the nail kegs. However, due at least to the small numbers taken and the erratic nature of the sizes of the catches in the nail kegs, these records are con-

sidered inferior to those of the cattle barns and sheds. The number present per barn or shed per inspection at the 0.1-mile distance was 4963, and the decline in numbers was very marked as the distance from the breeding areas was increased to 1 mile, only 250 being present per barn or shed per inspection at that distance. At 1.5, 2.0, and 3.0 miles very few were present. Likewise, at the 0.1-mile distance, there were 9.5 present per keg per inspection, and the decline in numbers as the distance increased is of much the same order

TABLE 1

*Densities of Female Anopheles quadrimaculatus as Measured by Estimates Made in Cattle Barns or Sheds and Collections from Nail Kegs at Varying Distances from the Sources of Production**

DISTANCE FROM BREEDING SOURCE IN MILES	BARN OR SHED		NAIL KEGS	
	Average number per inspection	Relative density†	Average number per inspection	Relative density†
0.1	4963	100.0	9.5	100.0
0.5	2506	50.5	3.0	31.6
1.0	250	5.0	0.4	4.2
1.5	10	0.2	0.2	2.1
2.0	4	‡	‡	
3.0	3	‡	‡	

* Four stations, each composed of a cattle barn or shed and 2 nail kegs, were used to establish the data for each distance. The records involve, generally, 17 inspections of each station during the summer. Most of the inspections were made by the junior writer, but some were made by the senior writer and Mr. Harvey L. Chada.

† Density expressed as a percentage of that existant at the source of production (0.1 mile distance), the latter density being taken as 100 per cent.

‡ Less than 0.1.

as that for the barn and shed data. It is apparent that the records obtained by the one method fully corroborate those obtained by the other.

Expressed on a percentage basis at the 0.5-mile distance, only 50.5 per cent as many *A. quadrimaculatus* were present as at the 0.1-mile distance. Actually, one of the stations listed as a 0.5-mile station (Location No. 14, figure 5), was only 0.3 mile distant from the production source and, for other reasons, it was a very favorable location. Therefore, the large numbers present there served to augment considerably the otherwise lower figure for the 0.5-mile distance. The 1.0-mile stations had only 5.0 per cent as many as did the 0.1-mile stations, and the 1.5, 2.0, and 3.0-mile stations showed increasingly smaller percentages.

Considering the data of table 1 from the purely biological standpoint, it is obvious that this mosquito has a relatively short distance of movement. There is, naturally, a great dispersion of numbers as the distance from a breeding source is increased. The size of the sample (speaking in terms of the number of stations used at the different distance zones) should, therefore, be increased proportionally to the increase in the area of one zone as compared to another to give a true picture of movement to each distance. In this study, due particularly to the nature of the terrain with respect to the breeding areas

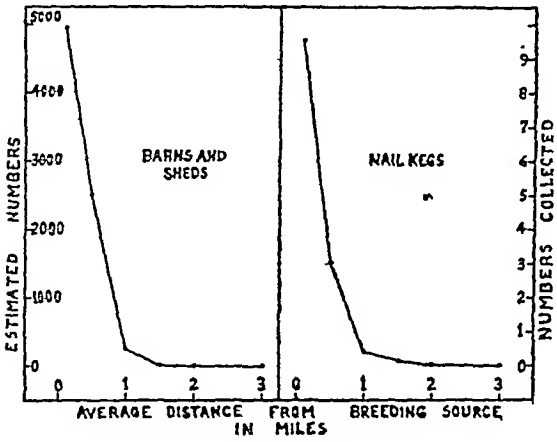

FIG. 2. Graphic presentation of the data in table 1, showing the densities of female *A. quadrimaculatus* at varying distances from the sources of production.

and to the unavailability of additional stations, this was not done. However, considering the nature of the terrain and the conclusiveness of the data along with this dispersion factor, there is no reason to believe that the graphic presentation of figure 2 is not a reasonably accurate picture of the movement of this species from its sources of production. Also, considering the question from the malarial rather than from the purely biological aspect, it is the numbers that have reached a given distance per unit area (density) rather than the total numbers having reached that distance, irrespective of that area, that is important.

An average of 250 female *A. quadrimaculatus* per barn or shed per inspection is obviously a dangerous density of this species. This was the number found at the 1-mile distance. Smith

(1942), reporting upon work in a malarious area for the Tennessee Valley Authority, considered the presence of 109 *A. quadrimaculatus* per barn per inspection to be a high density. The numbers of females of this species taken at the 1-mile distance were sufficient to warrant the conclusion that most of these came from the breeding areas under consideration; furthermore, that when production in a given area is *very heavy* this species may find its way as far as 1 mile, or slightly beyond, in numbers which could constitute a potential definitive host reservoir sufficient for the propagation of malaria. It may be said, also, that probably at least half of those found at the 1.5-mile distance (10 per barn or shed per inspection) came from the breeding areas under study. These flights up to 1 mile and beyond, contrary to the flights of 2.25 and 2.50 miles, as described by Eyles and Bishop (1943), were made despite the fact that cattle barns and homes, both with abundant sources of blood, were available at much shorter distances. Such movement from very large, heavily-producing, breeding areas to a distance of slightly beyond 1 mile may be sufficient to necessitate at least reasonable surveillance in the anti-malaria campaign. This study, however, fully confirms the conclusions of other authors that *A. quadrimaculatus*, in the great majority of situations where it is an important vector, does not move in significant numbers more than 1 mile from its breeding places.

PRODUCTION OF THE SPECIES IN THE CENTER UNDER STUDY

In order to establish the high productivity of this species in this study-locality, table 2 and figures 3, 4, and 5 are presented. Figure 3 shows *A. quadrimaculatus* resting on the walls of a section of a cattle barn in the St. Georges Basin area in September, 1941. The estimate made by the senior writer of about 40,000 of this species in this shed at a single inspection that year was reported by Stearns and Lynch (1942). Inspections of other barns and sheds during the period of this study (summer of 1942) have revealed as many as 15,000 to 25,000 on numerous occasions, and concentrations greater than that here shown (figure 3) have been fairly common. As previously stated, two large breeding areas, totalling nearly 1500 acres, constitute the productive center under study.

Figure 4 is a view of females of this species over-wintering in a vegetable cellar (the photograph taken on January 19, 1943). Approximately 10,000 were present in this same cellar about December 1, 1941; Huffaker (1942). Hinman's (1934) report of very large numbers of over-wintering females of this species was the only previous record of such large numbers, and W. V. King's remark, as a part of the discussion following Hinman's paper, shows clearly that such concentrations of over-wintering females are very unusual. Huffaker (1942) noted that, although

TABLE 2

*Estimated densities of female A. quadrimaculatus in the four cattle barns or sheds closest to the St. Georges Basin (near Fort duPont, Delaware) during the summer of 1942**

LOCATION	DATES OF INSPECTIONS																	TOTALS
	May		June			July					August			September				
	19	26	9	19	26	3	9	16	22	28	7	12	26	5	14	22	28	
No. 13 (Thorpe)...	63	313	4,013	4,624	5,502	17,425	3,653	16,321	9,533	15,823	2,873	4,407	1,728	5,123	1,247	393	192	93,233†
No. 14 (Myrre)		985	6,396	3,605	10,875	21,060	6,150	6,596	7,941	15,259	12,769	6,344	2,336	9,221	4,815	4,784	434	119,570‡
No. 19 (Denison)..	10	7	4,259	4,829	16,280	21,468	17,583	17,990	18,771	25,814	13,992	11,855	11,765	17,719	9,467	5,832	0	197,641§
No. 20 (Price).....	12	279	2,390	1,508	2,509	2,349	449	914	791	4,646	1,005	1,423	298	759	765	458	12	20,567¶
Totals.....	85	1584	17,058	14,566	35,166	62,302	27,835	41,821	37,036	61,542	30,639	24,029	16,127	32,822	16,294	11,467	638	431,011
Average....	28	396	4,265	3,642	8,792	15,576	6,959	10,455	9,259	15,386	7,660	6,007	4,032	8,206	4,074	2,867	160	

* See text for an explanation of the method used in arriving at estimates.
† Represents 82 per cent
‡ Represents 96 per cent.
§ Represents 85 per cent.
¶ Represents 81 per cent of the total mosquito estimate, males and females.

FIG. 3. FEMALES OF A. QUADRIMACULATUS IN A CATTLE SHED NEAR FORT DUPONT, DELAWARE

FIG. 4. FEMALES OF A. QUADRIMACULATUS OVER-WINTERING IN A CELLAR NEAR FORT DUPONT, DELAWARE

very large numbers of this species had entered hibernating quarters in this area in October and November, 1941, only a small number, 2 to 4 per cent, survived until the following spring. It was observed that, contrary to the condition in summer, females of *A. quadrimaculatus* in cold, hibernating, situations may not assume the erect resting position, but instead are found often in an almost recumbent position (parallel with the surface upon which they rest).

Regarding the expression, "area of high density," for this species, Smith (1942), as previously stated, considered the presence of 109 *A. quadrimaculatus* per barn per inspection as an indication of such an area. Compared to the Delaware records, this is a low density, but compared to

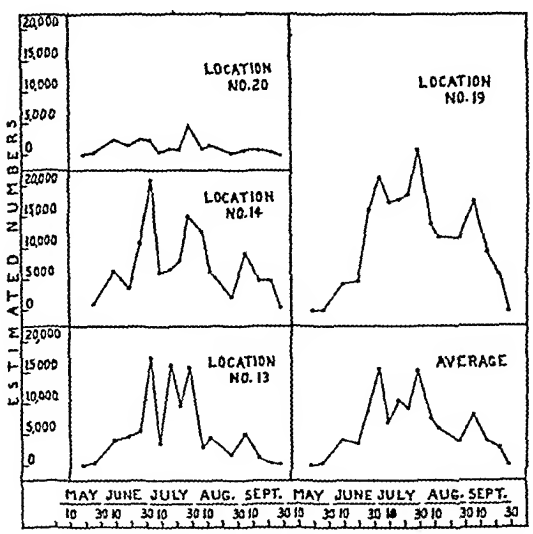

FIG. 5. Graphic presentation of the data in table 2, showing the densities and seasonal fluctuations in abundance of female *A. quadrimaculatus* in the four stations nearest the St. Georges Basin.

his other group might be looked upon as high. On the other hand, Horsfall (1942) caught in the neighborhood of 1500 of this species with a single New Jersey light trap on each of several different nights. Since it has been shown by both Horsfall (1943) and Huffaker and Back (1943) that this trap may be a very inefficient means of capturing this species (100 per night per trap was a maximum figure for the Fort duPont, Delaware, area), Horsfall's record of 1500 is evidence of exceptionally high density or else exceptional efficiency of the trap on those particular nights.

Breeding of *A. quadrimaculatus*, in the Fort duPont area, averaged 3.20 larvae and/or pupae per dip in July; 2.64, in August; and 1.66, in September, during the season of this study (a total of 2500 dips). Breeding was approximately comparable, though somewhat less, in 1941.

Figure 5 is a graphic presentation of the estimates of the numbers of female *A. quadrimaculatus* present at each inspection of the four stations closest to the St. Georges Basin during the period of this study. The average for the four stations is also included. These graphs were plotted from the data recorded in table 2.

SEASONAL FLUCTUATIONS IN NUMBERS

The data in table 2 and figure 5 establish a condition of heavy production of this anopheline and also may be viewed as a measure of seasonal fluctuations in the abundance of the species. The area considered herein is one which lends itself well to a study of the number of broods of *A. quadrimaculatus*, due not only to the extreme productiveness of the area but, as well, to the fact that the breeding situations are little affected by seasonal variations in rainfall. Hence, fluctuation in numbers may be taken as an indication of broods developed rather than purely the result of variations in rainfall and the consequent changes in the size of the breeding area. During the season of 1941, the senior writer found, from unpublished data, that a brood of *A. quadrimaculatus* seemed to have been produced at about 30-day intervals, one appearing in the early part of each of the months of July, August, September, and October. During that year, no observations had been made in May or June. The results, reported herein for 1942, are a verification of the 1941 observations. Examination of figure 5 will reveal brood indications during early June, early July, very late July (or very early August) and early September, 1942. Observations were discontinued before evidence of a brood in October became apparent. It may be noted that only one observation is contradictory to the existence of such peaks of abundance. This observation, on July 16 at Location No. 13, was not sufficient to change the aspect of the average curve. The June brood is certainly not clearly shown, but when it is considered that the over-wintered female population was very low, the evidence is adequate. In this connection, over-wintering females were observed to resume reproductive activities by the middle of March, and the first, male, *A. quadrimaculatus* was collected on May 19. Assuming, as did Hurlbut (1943), a very early brood too small to be quantitatively detected, about

the early part of May and another during November, it seems likely that 7 broods of *A. quadrimaculatus* are produced annually in Northern Delaware.

It is admitted that a much clearer and more dependable picture of brood production could have been obtained by making observations at frequent intervals (such as every four days). However, considering these results and the unpublished data of 1941, it is believed that these peaks do represent the production of broods. While Huffaker (1944) has reported that this species may complete all larval and pupal development in as little as 7.3 days under optimum conditions, a period of 30 days between broods in nature is not unexpected. Under optimum conditions of temperature and food supply, 9 to 10 days would be required from freshly-deposited egg to freshly-emerged adult. Also, from 4 to 6 days might be required for sexual maturation of the emerged female, the securing of a blood meal, and development of the ova and their deposition. Hence, under such conditions, 13 to 16 days would be required for the completion of a cycle. Considering the fact that food supply and temperature are probably never as favorable in an extensive breeding area as was the case in the controlled experiments of Huffaker (1944), it is expected that about 4 weeks would be required for the completion of a cycle under natural conditions in this area. This record is also in accord with the period between broods of this species as reported by Boyd (1930).

SUMMARY

A study is reported which confirms previously-published conclusions that, under normal conditions, *A. quadrimaculatus* does not move in significant numbers more than 1 mile from its breeding sources. However, an unusual condition is described where this species was observed to move 1 mile from its breeding areas in sufficient numbers to create a dangerous density—250 females per barn or shed per inspection. Near the sources of production, densities as high as 15,000 to 25,000 females per station per inspection are noted as very common, and one such record of 40,000 is included. In the study-locality (Fort duPont, Delaware) broods of this species are indicated at about monthly intervals during the summer season.

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TOXOPLASMOSIS MISTAKEN FOR HISTOPLASMOSIS IN A CAT

HENRY D. MELENRY

Errors sometimes creep into medical literature, and are subsequently quoted as facts in textbooks and other publications, thus encumbering the literature with misstatements which it is difficult to correct. For this reason, I venture to make the following correction as soon as possible. No criticism is intended of the authors quoted since the original error was caused by lack of experience with the parasites involved.

In the paper entitled, "Spontaneous Histoplasmosis Occurring in a Dog" by William P. Callahan, Jr., published in the AMERICAN JOURNAL OF TROPICAL MEDICINE for November 1944, (1) the author quoted Summerhill as having identified *Histoplasma* in the mesenteric lymph nodes and lungs of a cat. This statement by Summerhill was made in the discussion of my presentation of Histoplasmosis before the New York Pathological Society (2). At my request Summerhill sent me sections from this cat. The parasites, which were in large mononuclear phagocytes and in smooth muscle cells, did not have the appearance of *Histoplasma capsulatum*. I sent the sections to Dr. E. E. Tyzzer and Dr. David Weinman of the Harvard Medical School who identified the parasites as *Toxoplasma*. Meanwhile Dr. Summerhill had sent some of the tissues from the cat to the Cornell Veterinary School where a diagnosis of *Toxoplasma* was made. The case was reported by Olafson and Monlux in the Cornell Veterinarian for April 1942 (3).

REFERENCES

1. CALLAHAN, W. P., JR.: Spontaneous histoplasmosis occurring in a dog. Amer. Jour. Trop. Med., 24: 363–366, 1944.
2. MELENEY, H. E.: Histoplasmosis. Proc. N. Y. Path. Soc., 48–49, 1940–1941.
3. OLAFSON, P., AND MONLUX, W. S.: Toxoplasma infection in animals. The Cornell Veterinarian, 32: 176–190, 1942.

THE AMERICAN SOCIETY OF TROPICAL MEDICINE

FORMER PRESIDENTS

Thomas H. Fenton (deceased) 1904–1905
Roland G. Curtis (deceased) 1906–1907
James M. Anders (deceased) 1908–1909
W. C. Gorgas (deceased) 1910
W. S. Thayer (deceased) 1911
Joseph F. White 1912
Edward R. Stitt 1913
Richard P. Strong 1914
Charles F. Craig 1915
Milton J. Rosenau 1916
Bailey K. Ashford (deceased) 1917
C. C. Bass 1918
Henry J. Nichols (deceased) 1919
John M. Swan 1920
Victor G. Heiser 1921
George Dock 1922
Allen J. Smith (deceased) 1923
Samuel T. Darling (deceased) 1924
Joseph F. Siler 1925
George C. Shattuck 1926
Charles S. Butler (deceased) 1927
William E. Deeks (deceased) 1928
Kenneth M. Lynch 1929
Sidney K. Simon (deceased) 1930
Frank Smithies (deceased) 1931
George R. Callender 1932
Frederick F. Russell 1933
Edward B. Vedder 1934
Henry E. Meleney 1935
Herbert C. Clark 1936
Mark F. Boyd 1937
Alfred C. Reed 1938
Louis L. Williams 1939
Thomas T. Mackie 1940
Ernest Carroll Faust 1941
N. Paul Hudson 1942
Wilbur A. Sawyer 1943
R. E. Dyer 1944

COMMITTEES

(Chairmen are listed first)

Membership: E. G. Hakansson, P. F. Russell, W. H. Wright.

Award of the Walter Reed Medal: M. F. Boyd, H. E. Meleney, C. F. Craig.

Bailey K. Ashford Award: J. S. Simmons, R. A. Lambert, G. C. Shattuck.

Charles F. Craig Lecture: M. H. Soule, E. I. Salisbury, W. H. Taliaferro.

Honorary Membership: G. C. Shattuck, H. C. Clark, N. P. Hudson.

Program: J. S. D'Antoni, C. F. Craig, R. B. Watson.

Teaching of Tropical Medicine: H. E. Meleney, E. C. Faust, T. T. Mackie, P. Morales-Otero, H. W. Brown.

War and Post-War Tropical Medicine: A. J. Warren, L. T. Coggeshall, E. H. Hinman, N. H. Topping, O. R. McCoy.

REPRESENTATIVES TO OTHER ORGANIZATIONS

American Foundation for Tropical Medicine: E. C. Faust.

American Society of Parasitologists: C. G. Huff.

American Association for the Advancement of Science: H. C. Clark, E. C. Faust.

Division of Medical Sciences of the National Research Council: H. E. Meleney (for a period of 3 years from July 1, 1944).

TRANSACTIONS

Fortieth Annual Meeting

Meeting in St. Louis, Missouri, in conjunction with the National Malaria Society and the American Academy of Tropical Medicine, as guests of the Southern Medical Association, November 13–16, 1944.

Business Meetings

The Minutes of the Council Meeting

The annual business meeting (which was preceded by the annual dinner) of the Officers and Council of the Society was held at 6:30 p.m. on Tuesday November 14, at the Statler Hotel. Those present were: Doctors G. R. Callender, L. T. Coggeshall, J. T. Culbertson, J. S. D'Antoni, R. E. Dyer, J. F. Kessel, T. J. LeBlanc, O. R. McCoy, W. A. Sawyer, J. S. Simmons, A. J. Warren and R. B. Watson. Those absent were Doctors H. W. Brown and C. F. Craig. Former past presidents not presently officers or councilors who attended the meeting included Doctors M. F. Boyd, H. C. Clark, E. C. Faust, N. P. Hudson, H. E. Meleney, G. C. Shattuck and E. B. Vedder. Dr. J. T. Culbertson represented the American Society of Parasitologists. The Society had the pleasure of having one of its honorary members, Brigadier N. H. Fairley, present at this time. The president, Dr. W. A. Sawyer, presided.

1. The minutes of the previous meeting, November 15, 1943, were accepted as published in the March, 1944, issue of The American Journal of Tropical Medicine.

2. The president appointed Doctors LeBlanc and Dyer as the Resolutions Committee.

3. The Secretary's report was presented as follows:

Membership: The total active membership of the Society as of November 14 is 1213, as compared with 952 on approximately the same date in 1943. The increment and other changes are as follows:

4 deceased
3 resigned
15 delinquent in 1944

240 new members were added during the year, and the names of 43 others will be proposed for Council approval at this meeting, making a total of 283 new members in 1944.

At the present time the Society has 4 Emeritus and 20 Honorary members. In addition (and in addition to subscriptions by libraries, institutions, etc.) 14 non-members subscribe to The American Journal of Tropical Medicine. The Society thus carries a total of 1,251 names of individuals and organizations on the books, as compared to 987 at the end of 1943.

At the present time 71 members are delinquent 2 years and 205 are delinquent 1 year.

Certain details concerning the membership are of interest:

There are 691 non-military members, of whom 519 are within and 172 outside of the continental United States.

There are 522 members in service, of whom 342 are now stationed within and 180 outside of the continental United States.

There are 120 members "in status quo," of whom 2 are missing in action (William A. Hutchinson and Lewis T. Stoneburner, III) and 16 are in areas in which mail service has been suspended. The remaining 102 are members whose letters or publications have been returned and for whom no forwarding addresses have been supplied.

The roster of new members approved during the year and the roster of members to be approved at this meeting appear immediately following this report.

Those who resigned from active membership during 1944 are:

Susan G. Smith
Victor G. Heiser
Henry B. Webb

With regret the names of the following members are listed as having died during the past year:

Chas. S. Butler
Norman Epstein
John D. McBrearty
Archie S. Woods

Two of these were killed in action, Norman Epstein and John D. McBrearty. Letters of condolence are sent to the families of deceased members whenever the secretary's office is notified or learns of the death, and letters are also sent under the same circumstances to the families of men listed as missing in action.

Circular Letters: The following circular letters were sent out in 1944 to the Officers and Council:

January 7, announcing the increase in the allotment of paper for THE JOURNAL. June 2, announcing the date of the meeting and location. July 11, announcing that the National Research Council wished a member of the Society as representative in the Division of Medical Sciences, and enclosing the recommendations of the Committee on War and Post-War Tropical Medicine for comment. October 7, announcing the selection of Lowell T. Coggeshall to present the Ninth Charles Franklin Craig Lecture. Circular letters listing names of new members for approval were also sent March 2, April 12, August 1, and October 7.

Action of the Council by Correspondence:

(a) Approval of the names of 240 applicants for membership in the Society during 1944 (3/2, 4/12, 8/1 and 10/7).

(b) Naming Dr. H. E. Meleney as representative of the Society to the Division of Medical Sciences of the National Research Council for a 3-year period beginning July 1, 1944 (8/1).

(c) Approval of changing the annual Council Meeting to Tuesday night instead of Monday (7/11).

(d) Approval of inviting former Presidents of the Society to the Council meeting (7/11).

(e) Approval of defraying traveling expenses to the meeting of the Editor of THE JOURNAL, Colonel Charles F. Craig (7/11).

(f) Comments on recommendations of the Committee on War and Post-War Tropical Medicine (7/11).

(g) Approval of defraying the expenses of furnishing 200 copies of TROPICAL MEDICINE NEWS to malaria units overseas for the remainder of 1944 (8/1).

(h) Approval of the selection of Brigadier General James S. Simmons as recipient of the Walter Reed Medal (10/7).

During the year approximately 500 changes of address reached the Secretary's office, and this circumstance, combined with the large increase in membership, made it imperative to purchase for the Society some type of addressing machine, it being no longer practical to address bills and other communications by hand. With the approval of the President, an addressing machine was purchased, at a cost of $124.88.

The increase of membership and the additional work connected with this increase make it necessary for the Secretary to request an additional allotment for secretarial assistance. It is proposed that this allowance be raised from $20.00 to $50.00 per month.

The Secretary desires to express his sincere appreciation for the excellent cooperation shown by the Officers, Council and members of the Society during the past year. Special thanks are due all committee chairmen for their competent work, particularly the Chairman of the Membership Committee, Colonel Callender, and the members of this committee. The exigencies of the times have frequently made the execution of plans and the performance of even routine duties difficult, but the burden has been materially lightened by the cooperation of all concerned.

Attention is again called to the cooperation of the Southern Medical Association, through its Secretary-Manager, Mr. C. P. Loranz, with this Society.

ROSTER OF NEW MEMBERS APPROVED IN 1944

Abraham, Kurt
Adams, Deane Taylor
Aggeler, Paul Michael
Aguiar, Haroldo Werueck
Alberts, Irving Lester
Allen, Chester D.
Andrus, Willard B.
Ash, James Earle
Bauer, Frank L.
Bayrd, Edwin Dorrance
Beaver, Paul C.
Benavides, Juan
Berk, Morton Semur
Bernstein, Maxwell Robert
Bettle, Ronald Austin
Bews, Donald Cameron
Black, Sidney
Bolona, Francisco Enrique
Bonnell, George Harrison, Jr.
Booth, Carl Blackstone
Borer, Raymond John
Brady, Melvin
Breslow, Lester
Brooke, Marion Murphy
Brown, Omar J.
Browning, James S.
Buchenholz, Bruce Adolph
Bunting, John James
Burge, Martin H.
Burlingame, Paul L.
Burrett, John B.
Campbell, Eugene P.

Carbel, Tomas Jorge
Carter, Chapel E.
Casis-Sacre, Guillermo
Castro-Estrada, Santiago
Cataldo, Charles J.
Chandler, Lawrence U.
Cheevers, Owen James
Clapp, Ralph Sheldon
Coatney, G. Robert
Connelly, Michael Edward
Cooley, Samuel Studdiford
Cordi, Joseph Michael
Craig, Gladys Marie
Crawford, Alfred R.
Cronin, John Francis
Cross, Joy Barnes
Dain, Harvey Joshva
Davis, David Sidney
Davis, Edward Thomas, Jr.
Dettmann, Norbert Frederick
de Vasconcellos, Carlos
Dwork, Kermit Gordon
Ellis, Daniel W.
Evans, Robert Sherman
Fallis, A. Murray
Farrier, Robert Claude
Fernandez, Fernando Lopez
Fitts, Ralph Lamar
Fitzgerald, Edward Murray
Fletcher, Orlin Kenyon, Jr.
Foster, John Wesley
Franz, Karl H.
Fremont-Smith, Frank
Freundlich, Charles Gilbert
Frommeyer, Walter Benedict, Jr.
Furze, William Everett
Galup, Cesar
Gault, Edwin Sartain
Geiss, George William
Giffen, Horace K.
Gilman, Lloyd C.
Gilmore, Ray S.
Glascock, Harold Winfield, Jr.
Glorioso, John Alexander
Goldschmidt, Myer
Greene, Clyde Cornelius, Jr.
Griffin, Angus MacIvor
Handelman, Eugene Victor
Harrison, Rollie M.
Haskin, Aaron Henry
Havel, Thomas Earl
Heller, Elwyn L.
Henderson, Edward Ellice
Hiller, Carl R.
Hobbs, Elmer Theodore
Hockenga, Mark T.
Hodges, John Hendricks
Hoey, Charles F.
Hollands, Robert A.
Holmes, Robert H.
Holshouser, Charles A.
Hoon, James Richard
Horowitz, Jules Joseph
Jouse, Reginald Kimball
Howard, Ralph S., Jr.
Huffaker, Carl B.
Huntington, Robert W.
Huntley, Leslie Loran
Jones, Jack Colvard
Kannapel, Allen Robert
Kapernick, John Sturat
Kaplan, Arthur A.
Kaufman, Carl Kalman
Kaufman, Julius R.
Kelley, Joseph Francis
Kent, Donald Frederick
Kerr, Herbert H.
Kimball, Stockton
Kincov, Jacob
Kingsboro, Wilson Schwab
Kinsella, Ralph A., Jr.
Kirk, Warren Matas
Knies, Philip Thomas
Knight, Kenneth
Knott, James I.
Knott, Norman Laverne
Kossuth, Louis Caspar
Kozloff, Henry
Kroungold, Milton Leonard
Lackey, Marvin Alfonzo
Lagorte, Salvator
Lawler, Francis Cornelius
Lawton, Alfred H.
Leeds, Alexander Bartholomew W.
Lenke, Sidney Edward
Levin, Noah Bernard
Link, Richard Bleecker
Lloss-Garcia, Javier
Lovshin, Leonard L.
McArthur, Charles E.
McClosky, Ben Martin
McCoy, Hulburt C.
McKenna, Richard Donald
McLean, David William
MacDonald, Ogilvy James Scott
Manley, Joseph W.
Marshak, Irving J.
Marthouse, Stephen John
Martinez M., Ricardo A.
Maxwell, Elmer S.
Maxwell, Thomas
Meakins, Jonathan Fayette
Meza, Ruben Aguilar
Miller, Hubert Wainright
Miller, William Lindsay
Mitchell, Howard Fair

Mohr, John Luther
Mollohan, Cecil Spencer
Montero, Enrique
Moran, Donald Bernard
Morgan, Edward H.
Nicholas, Charles Andrew
Nigrelli, Ross F.
Norris, George Loren
Numainville, Leon Joseph
O'Brien, Henry R.
Ogryzlo, Metro Alexander
Oransky, Marvin
Osterlin, Ernst Julius
Parks, William Craig
Pearson, Stanley Michael
Pequeno, Eduardo Aguirre
Perkins, Jack F.
Perlman, Frank
Peters, Bruno Joseph
Petry, William A.
Pisani, Joseph Michael
Pollard, H. Marvin
Porter, Richard Janvier
Porter, William Lawrénc
Portuondo, Bonaventure Charles
Pugliese, Frank Anthony
Reeves, William Carlisle
Rein, Charles R.
Reiner, Ralph Everett
Rendtorff, R. C.
Rifkin, Harold
Roach, Carroll E.
Rosen, David
Rosen, Robert Leon
Roth, Herman W.
Ryon, William Albert
Saffold, John Henry
Sager, Robert V.
Sanchez, Francisco R.
Saracho-Lopez, Eduardo
Sather, George A.
Scaringi, Joseph
Schoene, Robert Henry
Schopick, Louis E.
Schroder, Jack Spalding
Seferlis, Louis S.
Shachtman, Joseph M.
Sheintoch, Hyman Rock
Silva-Pena, Fernando
Simmons, Robin Everett
Simpson, David Bertrand
Smith, Victor H.
Soberon y Parra, Galo
Sodeman, William Anthony
Staab, Frederick David
Stein, Robert Jacob
Steinberg, Arthur
Stewart, M. A.
Stillerman, Maxwell
Stimpert, Fred Dewey
Stone, Robert Edwards
Storch, Sidney
Stotts, Charles Stephen
Stump, David James
Talmage, Walter Raymond
Tavares, John M.
Taylor, Carl E.
Tovar, Raul Mancera
Trapido, Harold
Trewhitt, Madison S.
Trice, William W., Jr.
Tyson, Edward Boileau
Uribe, Luis C.
Van Buskirk, Kryder E.
Vandiviere, Stuart Pitner
Velez V., Gabriel
Wakefield, Howard
Wakim, Khalil Georges
Walker, J. Henry
Ward, Helen L.
Watt, James
Wellens, Stanley Lewis
Wenger, Don S.
Whitmore, Eugene Randolph
Wilder, Robert Lawson
Williams, Roger W.
Williamson, Lee
Wilson, Donald Robert
Wilson, Ira Herman
Yeager, Clark Harvey
Young, Viola Mae
Zivin, Israel

Listed below are the new members who have been approved by the membership committee but upon which the Council action has not been taken.

Allison, Thomas D.
Armistead, George C., Jr.
Chess, Stephen J.
Clark, Sidney B.
Daniels, Philip B.
Earle, Kevin V.
Ewing, Ben E.
Foertsch, Frederick E.
Galvao, Augusto L. A.
Hannum, William Y. C.
Hulsey, John McAfee, Jr.
Icenogle, Grover D.
King, Edward S.
Kohler, Carl W.
Lampesis, Peter T.
Larsh, John Edgar, Jr.
McConnell, Graham S.
Manning, John B., Jr.

Master, Brooker L.
Miller, Albert J.
Min, Thomas S.
Nickman, Emanuel H.
Nunez, Bernard E.
Pasquala, Thomas Louis
Pearce, John Y.
Raven, Clara
Rexer, William F.
Roop, Donald J.
Rosenberg, Saul
Saylor, Lawrence W.
Seabury, John H.
Slate, Joseph Esmond
Snapp, Robert H.
Stahl, William C.
Stephenson, Orlando K.
Stover, Lee
Thompson, Paul Everett
Tobias, Norman
Van Pernis, Paul A.
Walker, Douglass W.
Winer, Nahum J.
Winn, Paul F.
Wright, Richard B., Jr.

4. The following action was taken on the Secretary's report:

(a) 43 new members were elected by the Council.

(b) 15 members delinquent in dues were dropped from the rolls.

(c) Resignations submitted by 3 members were accepted.

(d) The deaths of 4 members were noted with deep regret.

(e) The sum of $50 per month was voted for secretarial assistance.

5. The report of the Treasurer was read (as follows) and accepted:

CHECKING ACCOUNT

	Receipts		Disbursements	
Balance on hand, November 7, 1943.	$636.02			
Membership dues...	4819.58			
Williams & Wilkins Company for profit on Volume 23 (1943) of The Journal.....	1400.33			
Checks not cashed by members (1943)...	4.60			
Refunds of exchange paid—year 1943...	0.96			
Total.....		$6861.49		
To Chas. F. Craig, Editor of The Journal, for secretarial assistance...			$150.00	
To Secretary, J. S. D'Antoni, for secretarial assistance 1943.....			125.00	
To Secretary, J. S. D'Antoni, for secretarial assistance 1944.....			220.00	
To Secretary, J. S. D'Antoni, for extra secretarial assistance 1944.....			39.00	
Postage.....			124.55	
Stationery and printing.....			113.50	
Telegrams and telephone calls.....			31.77	
Exchange paid bank on deposits for 1944			21.84	
Bank charges.....			4.88	
Refunds to applicants rejected for membership.....			30.00	
Extra copies of Tropical Medicine News sent to malaria units overseas			12.00	
Checks returned (not sufficient funds)...			15.00	
Purchase of addressing machine.....			124.88	
U. S. War Savings Bonds—Series F 1—$1000 (maturity value)	$740.00			
1—$500 (maturity value)	370.00		1110.00*	
Williams & Wilkins Company, Publishers, for The Journal.....			3782.00	
Incidental expenses..			33.99	
Total.....				$5938.41
Balance on hand, November 7, 1944.....			923.08	$6861.49

* The amount shown here for bonds is the purchase price. They will not mature for 12 years from date of purchase.

ASSETS

Balance in checking account.....		920.80	
Petty cash on hand..		2.28	
U. S. War Savings Bonds—Series F..		2442.00	
Addressing machine (purchasd 1944—cost) ...	$124.88		
Filing cabinet (purchased 1942—cost) ...	21.43	146.31	$3511.39

LIABILITIES

Due Williams & Wilkins Company for subscriptions to JOURNAL.....	160.00
Net Assets.....	$3351.39

Doctors R. B. Watson and O. R. McCoy were designated to audit the Treasurer's books and to approve them if correct.

6. The report of the Editor of THE JOURNAL (Colonel Charles F. Craig) was presented by the Assistant Editor, Doctor E. C. Faust, in the absence of the Editor:

With the appearance of the November number of THE AMERICAN JOURNAL OF TROPICAL MEDICINE, the twenty-fourth volume will have been completed and the most prosperous year in the history of THE JOURNAL will have ended. At the present time the subscription list is over three times as large as it was before the beginning of World War II, and it is still rapidly growing, thereby furnishing excellent evidence of the interest of the medical profession at this time in the subject of tropical medicine. It is to be hoped that this interest will not lessen with the ending of the war. There is good reason to believe that it will not, for thousands of our troops will continue to serve in tropical regions, and many will return to this country suffering from tropical diseases which will have to be diagnosed and treated. These circumstances will undoubtedly force the profession to maintain its present interest in the subject and our JOURNAL is a very potent help in this direction.

Owing to the shortage in paper it has been necessary to reduce the number of pages allowed THE JOURNAL by the publishers, but the reduction has not resulted in any reduction in the number of papers published during the year. This has been avoided by the use of narrower margins, double-column pages, and smaller type.

These changes have not improved the appearance of THE JOURNAL, but they have enabled us to give our readers as much material as in the past, and have greatly helped in the conservation of paper. The Editor believes that the changes meet with the approval of the Society, even though the format of THE JOURNAL may not be as attractive as before the war.

Owing to labor difficulties it has been impossible for the publishers to publish the issues of THE JOURNAL as promptly as in the past, but every effort has been made to do so.

At the present time THE JOURNAL is earning for the Society a substantial income, which will be reported by the Treasurer. This has been made possible by the new contract with the publishers, which was ratified last year and which is greatly to our advantage in many ways. At the present time, the financial condition of THE JOURNAL contrasts vividly with its financial condition some years ago, when it was indebted to the publishers to the extent of several thousands of dollars. In the meantime we have paid our debts and now are earning a good return on our investment. The present very satisfactory financial condition is due to the activity of our Secretary and Officers in securing new members, and to the fact that Government agencies have subscribed for many copies which are distributed to posts and hospitals throughout the world where our troops are operating.

The Editor desires to express his thanks to our Secretary, the Officers of the Society, our contributors, and to all others who have assisted in placing THE JOURNAL in the enviable position it now occupies in medical literature. Our thanks are due the Rockefeller Foundation for the payment for the printing of papers contributed by its members, and to the publishers, the Williams & Wilkins Company, for their constant courtesy and assistance.

7. The following action was taken on this report:

(a) It was received with a vote of sincere appreciation for the valuable work of the Editor.

(b) The sum of $200.00 was voted for secretarial assistance for the coming year.

8. The report of the Editor of TROPICAL MEDICINE NEWS (Joseph S. D'Antoni) was presented as follows:

As Editor of TROPICAL MEDICINE NEWS, which was ordered published as its official bulletin by the American Society of Tropical Medicine at the 1943 session, I am glad to report the successful carrying out of these instructions. Since the first issue appeared in February, 1944, the publication year will not end until the appearance of the December, 1944, issue, but there is no reason to anticipate during the remainder of the year any substantial alteration in the facts presented herewith.

As the members of the Society will recall, it was specified that the NEWS was to be entirely self-supporting, and an estimated financial statement makes clear that there is no likelihood of any deficit. In the unforeseen event that one should occur for the remaining issue for this year, it will be met by an increase in advertising fees for the coming year.

The NEWS was originally planned as a 16-page publication, of which 12 pages were to be text. From the very first issue, however, enough material has been available to make it a 24-page publication, of which 20 pages are text. The three original advertisers, G. D. Searle Company, John Wyeth and Brothers, Inc., and Eli Lilly and Company, were agreeable to meeting the extra cost of the additional pages, and there seems no reason why the same number of pages should not appear in all future bulletins.

The first two issues of the NEWS were printed in Baltimore, by the Waverly Press, which also prints THE AMERICAN JOURNAL OF TROPICAL MEDICINE. On the surface this seemed an excellent arrangement, but the NEWS is literally a bulletin of news, and delays in the mails and at the Baltimore end prolonged the process of getting out each of these issues over a 6-week period. Obviously this was very unsatisfactory, and arrangements were therefore made to print the NEWS in New Orleans. An excellent schedule has been worked out, and the NEWS now is ready for publication in a maximum of 18 days after it is given to the printer, aside from the added advantages of direct communication in regard to the many details which inevitably arise in any process of publication. The Waverly Press concurred in the necessity for the change and seemed relieved to be rid of one piece of work at this time.

That the NEWS has taken its place as an up-to-the-minute bulletin is proved, I think, by the numerous letters received concerning it from both members and non-members. Requests to be placed on the publication list (often accompanied by the statement that the subscription price will gladly be paid) have been received from 4 drug houses; 15 university and other libraries, 2 of which are outside of the country; 4 federal and state health departments; and several non-members now serving in the armed forces. The NEWS is not for sale, and these requests have been gladly met without cost. On July 6, 1944, a request was received from Major Oliver McCoy, of the Surgeon General's Office, for 200 copies of each issue for distribution among malaria units stationed overseas. The request was naturally flattering, but the budget of the NEWS did not permit the expenditure of the additional $12.00 per issue which these copies cost. The Council of the Society was therefore asked, and agreed, to finance the cost for the remaining issues of 1944, and the advertisers in 1945 will be asked to meet the additional expense. All of these requests are particularly gratifying because no attempt at all has been made to publicize the NEWS.

At the present time the mailing list of the NEWS, including the 200 copies sent to the Surgeon General's Office, totals 1,357 names.

Two pharmaceutical houses other than those now advertising in the NEWS have expressed a desire for advertising space in it, and I shall ask the membership of the Society to act upon this point. Although an additional sum of probably $700.00 per year would thus accrue to the treasury, I am not convinced of the wisdom of the inclusion of the additional pages of advertising, which would detract from the appearance of the bulletin and reduce the number of text pages.

Another matter upon which I should like an expression of opinion from the membership concerns the cover of the NEWS, which has come in for a good deal of criticism, always good-natured but nonetheless basically serious. In previous issues I have asked for suggestions as to a change, but have received none, and I should like the Council to discuss the matter at this time.

Finally, while as editor of the NEWS I have been much encouraged by the cooperation I have received from various members of the Society in the contribution of news items and scientific data suitable for publication in the form of brief notes, the response has not been general. It should be emphasized again that no editor can of himself publish an interesting bulletin, and the cooperation of the whole membership in this

regard would be greatly appreciated and would materially improve the contents of the NEWS.

9. The following action was taken on this report:

(a) It was accepted with special commendation.

(b) It was decided that the allocation of advertising space was under the jurisdiction of the Editor.

(c) It was decided that the present cover be retained until a more suitable cover be forthcoming.

10. The following committee reports were presented:

(a) The Committee on Honorary Membership. The name of Dr. Marshall A. Barber was proposed and accepted. Dr. Manuel Martinez Baez was nominated from the floor and the nomination was also accepted.

(b) The Membership Committee. This report is included in the report of the secretary (section 4a).

(c) The Walter Reed Medal Committee. This report is included in the report of the secretary (section h under "action of the Council by correspondence").

(d) The Charles F. Craig Lecture Committee. The selection of Lieutenant Commander L. T. Coggeshall to present this lecture was noted and the secretary was directed to extend the thanks and appreciation of the Society to the speaker.

(e) The Program Committee. (For complete program see pages 21–27.)

(f) The Committee of War and Post-War Tropical Medicine. The report was approved, and the secretary was directed to express the gratification of the Society over this excellent program which was approved by the U. S. Public Health Service and which will be sponsored by it.

(g) The Bailey K. Ashford Award Committee. The members of the committee were notified that this award will be available in 1945.

(h) The Committee on the Teaching of Tropical Medicine. The report was presented informally and accepted. This committee met earlier in the year with the committee on War and Post-War Tropical Medicine, and since the functions of this committee were adequately handled under the program outlined by the War and Post-War Tropical Medicine Committee, no further meetings were held.

(i) The Resolutions Committee. It was recommended to the Society that letters of appreciation be sent to the following individuals and organizations: Mr. C. P. Loranz, Southern Medical Association; Williams & Wilkins Company, publishers of THE AMERICAN JOURNAL OF TROPICAL MEDICINE; Colonel C. F. Craig, Editor of THE JOURNAL; Mr. J. C. Meacham, Hotel Statler; and Joseph S. D'Antoni, Editor of TROPICAL MEDICINE NEWS.

11. It was voted to continue affiliations with the Southern Medical Association and the National Malaria Society.

12. Appointments were made for the various committees and appear on page 2.

13. The Council voted to propose the following ballot of new officers to the Society: President-Elect, Brigadier General J. S. Simmons; Vice-President, P. F. Russell; Council for 4 years, E. I. Salisbury and E. G. Hakansson, and for 1 year to fill unexpired term of J. S. Simmons, L. T. Coggeshall; Editorial Board, O. R. McCoy, for 5 years to replace J. H. Kessel.

THE MINUTES OF THE ANNUAL BUSINESS MEETING

November 14, 1944, 6:30 p.m.

1. The minutes of the 1943 business meeting were accepted as published.

2. Transactions of the Council as set forth in items 3, 4, 5, 6, 7, 8, 9, 10 and 13 were approved.

3. Adjournment followed.

SCIENTIFIC SESSIONS

The first scientific session of the Society was called to order at 2 p.m., Tuesday, November 14, in Room 3B of the Municipal Auditorium, by President W. A. Sawyer, Washington, D. C. The program follows:

1. Immunity reactions in experimental relapsing fever, by Y. P. Chen, S. H. Zia and H. H. Anderson, University of California School of Medicine, San Francisco. Presented by Doctor Anderson. Discussed by Doctors Meleney and Soule.

2. Sporozoite-induced *Plasmodium lophurae* infections, by Richard J. Porter and Raymond L. Laird, School of Public Health, University of Michigan, Ann Arbor. Presented by Doctor Porter. Discussed by Doctor Huff.

3. Probable role of the cat flea, *Ctenocephalides felis*, in transmission of murine typhus, by J. V. Irons, S. W. Bohls, D. C. Thurman, Jr., and T. McGregor, State Health Department, Austin. Presented by Doctor Irons. Discussed by Doctors Faust, Hudson and Napier.

4. Rodents, rabbits and tularemia: Some zoological and epidemiological considerations, (originally, Rabbits and tularemia: Some zoological and epidemiological considerations) by William L. Jellison and R. R. Parker, Rocky Mountain Laboratory, U. S. Public Health Service, Hamilton. Presented by Doctor Jellison. No discussion.

WAR AND POST-WAR TROPICAL MEDICINE

5. The teaching of tropical medicine in the United States, by L. Everard Napier, Tulane University School of Medicine, New Orleans. Discussed by Doctors Barnes, Faust, Kellersberger and Shattuck.

6. Some epidemiological aspects of infectious hepatitis in the U. S. Army (by invitation), by Douglass W. Walker, Major, Medical Corps, AUS, Office of the Surgeon General, Washington. Discussed by Doctors Meleney and Soper.

7. Foreign quarantine in military traffic, by Phillip T. Knies, Lieutenant Colonel, Medical Corps, AUS, Office of the Surgeon General, Washington. Discussed by Doctor Hudson.

8. Wartime tropical medicine activities of the National Research Council, by Henry E. Meleney, New York University College of Medicine, New York. No discussion.

9. Activities of the Distributing Center for Parasitological Specimens during 1944, by George W. Hunter, III, Major, Sanitary Corps, AUS, Army Medical School, Washington. No discussion.

Discussion on War and Post-War Tropical Medicine by Doctors Dyer, Meleney and Salisbury, with Doctor A. J. Warren presiding.

JOINT SESSION WITH THE NATIONAL MALARIA SOCIETY

The second scientific session of the Society was called to order at 9 a.m., Wednesday, November 15, in Room 3A of the Municipal Auditorium, with Doctor G. H. Bradley, Atlanta, Georgia, President of the National Malaria Society, and Doctor Wilbur A. Sawyer, President of the American Society of Tropical Medicine, presiding. The program follows:

10. Use of atabrine in the treatment of malaria, by James A. Shannon, New York University College of Medicine, New York. Discussion opened by Doctor Henry E. Meleney.

11. Suppressive treatment of malaria in military forces, by Oliver R. McCoy, Lieutenant Colonel, Medical Corps, AUS, Office of the Surgeon General, Washington.

12. Preliminary report on imported malaria studies, by Martin D. Young, Joseph A. Moore, Frederick C. Ehrman, Trawick H. Stubbs, Newton F. Hardman, John M. Ellis, and Robert W. Burgess, U. S. Public Health Service, Columbia. Presented by Doctor Stubbs.

13. The effect of phenyldrazine on the pigeon strain of *Plasmodium relictum*, by W. B. Redmond and Grattan Crowe Woodson, Emory University, Atlanta. Presented by Doctor Redmond. Discussion opened by Doctors Hewitt and Coatney, of Memphis and Bethesda.

14. Factors influencing the uneven distribution of *Aedes aegypti* in Texas cities, by Asa C. Chandler, U. S. Public Health Service, Houston. Discussion opened by Doctor Usinger of Atlanta.

15. Report of an attack of blackwater fever subsequent to induced malaria, by S. F. Kitchen and G. G. Sadler, Rockefeller Foundation, Station for Malaria Research, Tallahassee and Florida State Hospital, Chattahoochee.

16. The detection of the *Plasmodia* of human malaria in blood films of fluorescence microscopy (by invitation), by Robert L. Metcalf, Tennessee Valley Authority, Wilson Dam.

17. Changes associated with acquired immunity in the malaria of lizards, by Paul E. Thompson, Tulane University School of Medicine, New Orleans.

18. Infections with blood and tissue stages of malarial parasites in relation to natural and acquired immunity, by Clay G. Huff and Frederick Coulston, University of Chicago, Chicago. Presented by Doctor Huff.

19. The inhibiting effect of pyridoxine on the activity of quinine and atabrine against avian malaria, by Albert O. Seeler, Merck Institute for Therapeutic Research, Rahway.

20. Medical shock in the pathogenesis of algid malaria, by B. H. Kean, Captain, Medical Corps, AUS, and Carl E. Taylor, Ancon, Canal Zone. Presented by Doctor Taylor.

The third scientific session was called to order Wednesday afternoon, November 15, at 2 p.m. in room 3B of the Municipal Auditorium, by President W. A. Sawyer. The program follows:

21. Interdermal and complement-fixation reactions elicited by various antigens in persons infected with *Onchocerca volvulus*, by John Bozicevich, Anthony Donovan, Luis Mazzotti, Francisco Diaz A., and Enrique Padilla, Pan-American Sanitary Bureau, Mexico, and Sanidad Publica, Guatemala. Presented by Doctor

Bozicevich. Discussed by Doctors Tsuchiya and Kellersberger.

22. *Onchocerc avolvulus:* A new stain for demonstration of microfilaria in tissues; observations on detailed anatomy and fate and distribution of the parasites in tissues, by William McKee German, University of Cincinnati College of Medicine, Cincinnati. Discussed by Doctors Hakansson, McCoy, Otto and Sandground.

23. Tests of mercury and antimony compounds in *Dirofilaria immitis* and *Litomosoides carinii* infections, by A. H. Lawton, F. J. Brady, A. T. Ness and W. T. Haskins, National Institute of Health, Bethesda. Presented by Doctor Brady. Discussed by Doctors Anderson, Andrews and Napier.

24. Presentation of the Walter Reed Medal of the American Society of Tropical Medicine to James S. Simmons, Brigadier General, USA, Office of the Surgeon General, Washington, D. C.

25. Localization of trivalent radioactive antimony following intravenous administration to dogs infected with *Dirofilaria immitis*, by F. J. Brady, A. H. Lawton, D. B. Cowie, H. L. Andrews, A. T. Ness and G. E. Ogden, National Institute of Health, Bethesda, and the Carnegie Institution, Washington. Presented by Doctor Lawton. Discussed by Doctors Anderson, Andrews and Napier.

26. Chemotherapy of human filariasis with pentavalent antimony compounds, by James T. Culbertson and Harry M. Rose, Columbia University College of Physicians and Surgeons, New York. Presented by Doctor Culbertson. Discussed by Doctors Anderson, Giffen, Napier, Vedder and Wright.

27. Experiments to determine potential mosquito vectors of *Wuchereria bancrofti* in the continental United States, by Walter L. Newton, Willard H. Wright, and Ivan Pratt, National Institute of Health, Bethesda. Presented by Doctor Newton. Discussed by Doctors Butler and Wright.

28. Immunity developed as a result of experimental *Necator americanus* infections in man, by G. F. Otto, Johns Hopkins University School of Hygiene and Public Health, Baltimore. Discussed by Doctor Hunter.

The fourth scientific session was called to order Thursday morning, November 15, at 9 a.m. in Room 3B of the Municipal Auditorium, with President-Elect, R. E. Dyer, Bethesda, Maryland, presiding. The program follows:

29. Preliminary report of a new trypanocidal agent: γ-(p-Arsenosophenyl)-butyric acid (by invitation), by Harry Eagle and Harold J. Magnuson, U. S. Public Health Service, Johns Hopkins Hospital, Baltimore. Presented by Doctor Magnuson. Discussed by Doctors Kellersberger, Salisbury and Sandground.

30. Survival time of trophozoites of *Endamoeba histolytica* and its practical significance in diagnosis, by H. Tsuchiya, Washington University School of Medicine, St. Louis. Discussed by Doctors Faust and Meleney.

31. Amebic hepatitis, by W. A. Sodeman and B. O. Lewis, Tulane University School of Medicine and U. S. Marine Hospital, New Orleans. Presented by Doctor Sodeman. Discussed by Doctors Coggeshall, Faust, Kessel, Lewis and Tsuchiya.

32. Early results of the treatment of African trypanosomiasis with two new arsenical preparations (melarsen oxide and 70A), by David Weinman and Karl Franz, Harvard Medical School and School of Public Health, Boston. Presented by Doctor Weinman. Discussed by Doctors Anderson, Kellersberger, Sandground and Tsuchiya.

33. The Ninth Charles Franklin Craig Lecture on tropical medicine: Malaria in the returning serviceman, by L. T. Coggeshall, Commander, Medical Corps, USNR, Marine Barracks, Klamath Falls.

34. Simultaneous vaccination against bacillary dysentery and cholera with toxoid-vaccine, by Oscar Felsenfeld and Viola Mae Young, Chicago Medical School, Chicago. Presented by Doctor Felsenfeld. No discussion.

35. Risk of attack in leprosy in relation to age at exposure (by invitation), by James A. Doull, Ricardo S. Guinto, Jose N. Rodriguez and Huldah Bancroft, Western Reserve University, Cleveland; American Leprosy Foundation, Leonard Wood Memorial; and Bureau of Health of the Philippines, Leprosy Section. Presented by Doctor Bancroft. Discussed by Doctors Kellersberger, Soule and Vedder.

36. The reaction of lepromin antigen in patients with sarcoid and tuberculosis, and in normal individuals, by George T. Harrell, Jr., and S. F. Horne, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem. Presented by Doctor Harrell. No discussion.

37. Blood studies in fifty-two cases of Hansen's disease (leprosy), by Carroll L. Birch, University

of Illinois College of Medicine, Chicago. Presented by Doctor Birch. No discussion.

The following papers were listed by title or were not presented because of the essayists' inability to be present at the meeting.

38. Twenty-five years of research work on tropical pathology in America, by W. H. Hoffmann, Finlay Institute, Havana, Cuba.

39. An inquiry into the growth factor or factors of certain blood and tissue flagellates, by Harry A. Senekjie and Ruth A. Lewis, Tulane University School of Medicine and Tulane Amebiasis Unit, National Institute of Health, New Orleans.

40. Opportunities for training and research in tropical medicine and public health in Mexico, by Manuel Martinez Baez and E. Harold Hinman, Salubridad y Asistencia, and Institute Inter-American Affairs, Division of Health and Sanitation, Mexico.

41. The organization and function of the tropical disease section at Moore General Hospital, by Harry A. Most, Captain, Medical Corps, AUS, Moore General Hospital, Swannanoa.

42. Simplified quantitative methods for hookworm control programs, by J. Allen Scott, University of Texas Medical Branch, Galveston.

43. Comparative yields of *Endamoeba histolytica* Organism *T* from soluble and insoluble ingredients of egg white in freshly prepared and stored medium, by Chas. W. Rees and Lucy V. Reardon, National Institute of Health, Bethesda.

44. Mal del pinto, by Eduardo Aguirre Pequeno, Monterrey.

Other Events

1. The annual luncheon of the Society was held Wednesday, November 15, at 12:30 p.m. The President of the Society, Dr. W. A. Sawyer, Washington, D. C., who was introduced by the President-Elect, Dr. R. E. Dyer, presented as his presidential address "The place of tropical medicine in international health." Former presidents were seated at the speaker's table, as were representatives from the Academy of Tropical Medicine and the National Malaria Society.

2. Well attended hospitality group sessions were held on November 13, 14 and 15 at 5 p.m. Informal talks were made at these sessions by Dr. W. A. Sawyer, who spoke on UNRRA; Dr. N. H. Fairley, who spoke on certain aspects of malaria, and Dr. H. C. Clark, who spoke on personal hygiene in the tropics.

3. The American Academy of Tropical Medicine held its eleventh annual dinner at 7 p.m., Wednesday, November 15, to which all members of the Society were invited. Dr. Malcolm H. Soule was toastmaster.

Colonel E. B. Vedder, Oakland, California, presented as his presidential address "The present status of tropical medicine and some future problems."

ERRATA

The name, "Henry D. Melenry" as author of the note entitled "Toxoplasmosis Mistaken for Histoplasmosis in a Cat" should be "Henry E. Meleney".

MALARIA AND FILARIASIS IN THE RETURNING SERVICEMAN

The Ninth Charles Franklin Craig Lecture[1]

L. T. Coggeshall[2]

From the U. S. Marine Barracks, Klamath Falls, Oregon

Received for publication February 6, 1945

The general problem of malaria as it exists in the returning serviceman is a familiar one to those interested in tropical diseases, at least in its broader aspects. More specifically, it is a disease present in large numbers of men and we are concerned with the task of reducing their latent infections and preventing malaria from gaining a foothold in receptive areas of this country. The basis of this report will be a discussion of the problem as seen in a group in excess of 3000 men assembled for care and observation at one place.

Since the station referred to is somewhat unorthodox as compared to other medical military installations, a word of explanation is in order. A little over a year ago it was the opinion of the Bureau of Medicine and Surgery, U. S. Navy, that a special installation for the care of malaria patients was justified. There were many reasons for this decision; first, because of the large volume of men involved; second, because the majority of these individuals were having repeated clinical breakdowns; third, they were only acutely ill for three to seven days during each episode, thus occupying valuable hospital beds urgently needed for other purposes; and finally, it was considered an ideal opportunity to assemble these malarial patients in one place for observation and study. Never in the history of this country has such a large group of men suffering from a single infection been returned to a clean area, without the opportunity for reinfection, where they can be retained as a body throughout their convalescent period. As these men are returned from overseas, they are immediately sent to Marine Barracks, Klamath Falls, Oregon, where they are carefully questioned as to their itinerary and malaria history, examined, and if physically able to travel, are given a month's furlough. The malariologist will probably question the wisdom of this last procedure, since it enhances the possibilities whereby malaria may establish itself in this country. This policy, in our opinion, is justified from the fact that already thousands of recurring cases have been dispersed by all military services through assignment, furlough or discharge to all states of the Union. There probably is not a community which has not received one of these servicemen suffering from this malady. Also there are relatively few areas in this country where the possibility of transmission can be excluded. Even if there were malaria-safe places it would mean the retention of these men until the last recrudescence has occurred—something no one can predict and many are now going into their third year of infection. Since the majority of cases seen in this country contracted their infection in Guadalcanal and adjacent islands late in 1942, one can easily visualize the difficulty of restricting a man who has served overseas two years, and especially in those who have hit the beaches on Guadalcanal, Tarawa, Eniwetok and Saipan with periodic malaria as a constant companion.

After their return from furlough, they were again examined and assigned to companies of 200 men each, where they can be closely followed and observed. An analysis of their malaria histories overseas reveals several interesting points. First, occasionally one encounters among the patients men from the South who have had previous malaria histories in this country and not in the too distant past. Of course these are few in number and it furnishes only inferential evidence on the possibility of the lack of cross immunity between American and South Pacific strains. Especially so since we do not see those men who have had malaria in this country and were exposed but did not acquire it overseas. The information is presented only as an observation for what it is worth.

An examination of the health records does not furnish an accurate picture of the species of plasmodia responsible for the initial infection, since many men were diagnosed and treated without the aid of blood smears, probably many blood

[1] Presented at the Fortieth Annual Meeting of the American Society of Tropical Medicine, at St. Louis, Mo., November 13–16, 1944.

[2] Captain (MC) U. S. Navy Reserve.

smears were misdiagnosed and many unrecorded. However, from the hundreds of records examined little doubt remains that in the field a high percentage were accurately diagnosed as falciparum malaria. Also, from other records and statements of competent medical officers, it is known that falciparum malaria was extremely prevalent in the South Pacific area in general and in some areas it predominated over the vivax variety. However, the exact ratios of the two are not known. Likewise, the percentage of men suffering clinical reactivations following the initial attack is not known because unit strength information is not available, replacements were frequent, there were high casualty rates and men were constantly being reinfected. However, some careful studies were made on small units where it was possible to obtain non-controversial information and although the data has been placed on the restricted list, it is possible to state that the percentage of recrudescences is greater than that commonly observed with vivax malaria in this country.

Most of the men on the station, among 1424 cases analyzed, 1335 or 95%, took suppressive medication before their initial attack. From the histories only one positive statement can be made in regard to suppressive therapy, either quinine or atabrine, and that is; a sufficient number regularly took either drug in the recommended dosage or in excess and it did not prevent malaria. The individuals were subjected to so many combinations of suppressive drugs and dosage regimes a critical analysis of their malarial histories is not possible, especially as to number of clinical breaks through while under suppressive therapy.

From the records on the toxicity of atabrine given as a suppressive drug, it can be stated that in some instances dosages of 0.3 gm. daily were tolerated for long periods without difficulty. The toxicity of this drug has been studied in man and animal under the auspices of the Army, Navy and National Research Council, and it is the consensus of opinion that the toxic properties of atabrine in the recommended dosage is of little consequence. As to acute toxicity, there is a record at Oak Knoll Naval Hospital (1) of a man who was known to have taken at least 7.5 grams of atabrine and probably considerably more (18.0 grams) in a three hour period with a suicidal intent. He went into shock, was stuporous in about four hours, then went into coma. Within 48 hours he was conscious and went on to complete recovery. There was no evidence of liver or other visceral damage. On the second day, his atabrine blood level was 475 gamma, which is only 25 gamma below the saturation level. It it unfortunate in one respect that the man did not have chronic malaria because a golden opportunity was lost to determine the effectiveness of atabrine as a cure when present at an excessive level. Brigadier N. H. Fairley (2), Australia, has seen a similar episode which resulted fatally. This gives a mortality figure of 50% in a series of two cases. Although these are extreme cases, the dosages with survival does indicate that a slight increase in recommended dosage would not be attended by serious results.

TABLE I

Number of previous attacks related to results of blood smear examinations taken upon admission

NUMBER OF PREVIOUS ATTACKS	BLOOD SMEAR NUMBER		
	Total	Positive smear	Negative smear
All cases	1,419	116	1,303
1 attack elsewhere	33	1	32
1 attack here	3	0	3
2–4 attacks	130	15	115
5–9 attacks	437	28	409
10–14 attacks	418	40	378
15–19 attacks	241	18	223
20–24 attacks	95	7	88
25–29 attacks	44	7	37
30–34 attacks	10	0	10
35 and over	5	0	5

When the men are transferred to the station, all those with a malarial history have a stained thick smear of their blood examined. A summary of the results of these examinations is shown in Table I. In 1419 individuals, 116, or 8.2% were found to be positive. This does not include those men who arrive with an acute attack. When the degree of parasitemia is compared to the number of attacks that occurred previous to arrival, it can be seen that no correlation exists. For example, 418, or 29% of all cases aboard have experienced 10–14 attacks and in this group we find 34% of the positive smears. Although the positive smears are somewhat greater in this group, the difference is not a significant one. Separate from this group and not shown on the table are the results of the men who have had clinical malaria on the

station, who are treated and then followed by twice weekly blood smears. In 1427 such examinations, 132, or 9.7%, are found to have circulating parasites. As expected, this figure is slightly higher than the 8.2% showing a parasitemia upon arrival on the station.

TABLE II

Number of previous attacks related to spleen and liver examinations

NUMBER OF PREVIOUS ATTACKS	SPLEEN AND LIVER EXAMINATIONS NUMBER			
	Total	Palp. spleen	Palp. liver	Non-palp. spl. and liver
All cases	1,419	55	14	1,349
1 attack elsewhere	33	0	0	33
1 attack here	3	0	0	3
2–4 attacks	130	1	3	126
5–9 attacks	437	15	4	417
10–14 attacks	418	18	2	398
15–19 attacks	241	11	3	227
20–24 attacks	95	3	1	91
25–29 attacks	44	4	1	39
30–34 attacks	10	2	0	8
35 and over	5	0	0	5
Inf. miss	3	1	0	2

TABLE III

Spleen and liver examinations related to original blood smear results

SPLEEN AND LIVER EXAMINATION	RESULTS OF SMEAR NUMBER		
	Total	Positive	Negative
All cases	1,419	116	1,303
Pal. spleen	55	5	50
Pal. liver	14	2	12
Both	1	0	1
Neither	1,349	109	1,240

1 Less than 0.5 of one per cent.

In spite of the high incidence of falciparum malaria reported in the previously referred to initial infections, not a single infection of this variety has been seen on our station. Nor have any falciparum gametocytes been recognized. The men have come directly to the station from overseas without selection. Undoubtedly, many of them have acquired mixed infections, but as the biological characteristic of falciparum malaria is not to relapse, they are not seen here. With stricter suppressive atabrine discipline, falciparum malaria is failing to make its appearance where there is every reason to expect it. Probably atabrine neither prevents nor cures this type of malaria but merely suppresses until it is finally eradicated by the defense mechanism of the body.

Also in the same table the number of previous attacks are shown. Since the information is gained by testimony, the numbers are not absolute, because after 8–10 recrudescences many men lose count of the exact number. However, in a group of this size the percentage of error is a small one. Of interest are three men who have experienced their first malarial attack on the station. Their

TABLE IV

Monthly relapse rate of personnel on station with malarial histories

MONTH	NUMBER OF MEN	NUMBER OF RECRUDESCENCES	RATE
June	29	2	6.9
July	184	19	10.3
August	411	30	7.3
September	569	66	11.6
October	802	89	11.1
Average			10.3

admission diagnosis was filariasis. One of the men had this first attack 8 months following withdrawal from the endemic area and discontinuance of suppressive atabrine. This has been the longest noted in any of many on our station, although there is another authentic instance of a 13 months' period without history of malaria overseas.

The complement fixation reaction as a diagnostic aid has been employed intensively in many military installations in the past two years. This test was first employed by Kingsbury (3) most successfully a decade ago when he employed infected vivax and falciparum blood as an antigen. It was discarded because this source of antigen was unsatisfactory. Eaton and Coggeshall (4), in 1937, showed that a non-human malaria parasite, *Plasmodium knowlesi*, could be used just as successfully and thereby excluded the chief disadvantage of obtaining antigen from ill human subjects. In therapeutic malaria induced by infected blood

where relapses practically never occur, the findings were very promising. We have used the test for the past several months in an attempt to detect hidden infections or to delineate the duration of the disease acquired in the South Pacific. There is no doubt as to its specificity, as a positive test indicates infection. However, in the majority of positive cases, proven by subsequent relapses, we have found that the test is an unreliable one, as it is too frequently negative in these known positives. These results have occurred with all the various types of antigens recently described.

As one observes the clinical picture in several hundreds of these repeating vivax infections, one is impressed by several points. The first is the absence of the picture of malaria cachexia so frequently described with chronic malaria. This is particularly true in the men who have been back a few weeks. Either malarial cachexia is only evident when associated with other infections and malnutrition or the manifestations as seen in South Pacific malaria are different than those encountered elsewhere. Likewise, splenomegaly is noted in less than 5% of the men, even after 20 to 30 recrudescences. Immediately after the acute attack, palpable spleens are present in about 8% of the individuals, most of them have barely palpable spleens, three finger enlargement has been the maximum. This information is summarized in Tables II and III. Anemia is also conspicuous by its absence, after six weeks at our station with mile high altitude, the red blood count averages 5,300,000. Except for a persistent headache in a few individuals there is nothing to indicate that an individual is suffering any discomfort between his acute episodes of fever.

When ill, the response to therapy is prompt, within 72 hours. This can be accomplished by administering orally 0.2 gm. atabrine every six hours and then 0.3 gm. daily for five days. Any greater amounts in a short time or prolongating therapy has had no influence on the subsequent relapse rate. Quinine behaves similarly and shows the same fundamental inability to effect a permanent cure. Plasmochin, now in general disrepute because of its toxicity, has not yet been shown to influence the relapse rate. Likewise, arsenicals or other heavy metals, sulfonamides, penicillin, dyes or other types of compounds are in general of little or no avail.

Among the individuals on the station, the recrudescence rate has averaged 10.3 per cent per month. This figure is obtained from the number with a malarial history and relapses each month (Table IV).

Considerable controversy has arisen as to whether the vivax malaria originating in the South Pacific area represents a strain differing from the same variety seen in this country. No immunological studies have been conducted on our station but there is considerable inferential data to support the thesis that they do differ. In the first place, the frequency of periods of clinical activity following the initial infection is far in excess of that seen in this or any other country. As shown in Table I, 57% of the men under observation have had in excess of 14 acute attacks, some of whom experienced as many as 40. This is in contrast to the picture observed in vivax malaria of the States, where it is unusual to have a fraction of this number of clinical breakdowns.

Also in the overseas group, one is impressed with the rhythm of the recrudescences. Many men can predict within a week when their next bout will occur. A common statement is that, "my malaria was much more regular than the pay check".

Another point of differentiation is the previously referred to lack of splenomegaly, high blood counts, general well-being, even in men with persistent parasitemia, and frequent recrudescences.

Not observed on the station, but another point of differentiation is that Negroes are very refractory to the vivax malaria of the United States. As a matter of fact, institutions using therapeutic malaria for C.N.S. lues resort to the quartan type. In the Pacific area, American Negroes were as susceptible to vivax malaria as whites. Butler and Sapero (5), (MC), U.S.N., conducted an epidemiological study on three battalions of colored troops from South Carolina who contracted malaria, and 90% of readmissions were diagnosed vivax malaria. Actually, the relapse rate for vivax malaria was slightly greater than in white troops serving in the same area. There is much more inferential evidence that there are strains within species of malaria plasmodia and that contracted in the South Pacific is the most tenacious yet encountered. If this impression is confirmed the greatest danger of introducing malaria into this country probably is not that sweeping epidemics will occur, but the implantation of a strain immunologically different from those indigenous to this country, and one that will incapacitate for long periods all those who contract it.

The activities of the station are concerned with the assimilation of information with considerable emphasis in investigation. For the most part it concerns evaluation of new therapeutic compounds, the results of which cannot be disclosed at the moment.

It is possible, however, to describe the program of therapy, although it is non-specific and has been in force only a few months, there is no doubt that much is being accomplished. When a man has returned from furlough he is placed on a full duty status. This includes military training, recreational activities and educational and vocational exercises. It is noted that the men gain weight, the red cell count and hemoglobin increases and there is a rapid improvement in their general physical condition. Approximately 85% of our men come from overseas and the remaining 15% have been transferred from continental hospitals or limited duty stations. Men who have been in this country for some time and only able to perform two or three hours' work daily soon are able to undergo a full day's activity.

The abuse of rest is very apparent during the convalescent period from this disease, as it is being observed in many other infections or disabilities, and certainly any man with malaria needs very little encouragement to make him a permanent mental and physical cripple. If, by careful study, the future course of these infections of several hundred men with malaria can be more clearly defined, it will be of real value to all those concerned, and during the course of these studies if the ideal drug should happen to be disclosed which would more rapidly terminate the course of this infection, the effects would be so far reaching that one would hardly be able to visualize its importance. These two possibilities remain in the future, but this accomplishment can be definitely stated at this time, namely, there is no doubt that the program designed to keep men occupied mentally and physically during their convalescent period from tropical infections is real preventive medicine in that it is preventing the inception of hundreds of cases of "hospitalitis". Thus, in the absence of curative drugs and with diseases that are gradually being eliminated by the defense mechanism of the host, our rationale of treatment is based on the concept that a physically fit body is equipped to throw off an infection more quickly than the one that is permitted to deteriorate mentally and physically by long periods of hospitalization.

Finally, there seems to be no doubt that even this South Pacific malaria is a self-limited disease and will eventually disappear. Actually, within a relatively short six months period, we are noticing that a curve representing the rate of recrudescences has a definite downward trend in this malaria that is still manifesting itself in the third year after inception, and a program of physical fitness is precipitating the fall of the curve.

FILARIASIS

When filariasis made its appearance among servicemen stationed in the South Pacific, a little more than a year and a half ago, it was a cause of considerable concern to medical men. This concern, more than anything else, had as its genesis the picture of elephantiasis seen in the natives, who have carried their infections throughout their life-time. Filariasis is especially prevalent in the Samoan area. Actually, it exists there in epidemic form, although it is not correct to refer to its existence as epidemic, even where the incidence is so uniformly high. Epidemics occur when large groups of uninfected populations immigrate into an infected area. This is what happened early in 1942, when several thousand American Marines were staging in British and American Samoa, Wallis and Ellice Islands. The causative organism is *Wuchereria bancrofti*, which is the most prevalent of the four species of the filarial organisms. The life history of this nematode worm is fairly well understood. It is transmitted to man by an infected mosquito, which injects sexually immature microfilaria into the blood stream. These embryos make their way to the lymphatic channels and thence to the lymph glands, where they mature into adult organisms. The male wraps itself around the female and fertilization occurs. After the female becomes gravid she expels microfilaria into the blood stream. The microfilaria are thus available to the bite of a susceptible mosquito. The microfilaria, in themselves, are non-pathogenic and non-infective to man. Actually, the circulating microfilaria can be transferred by transfusion to a normal individual without harm. They can only complete their development by passing through the mosquito and thence back to man. This is an important point from the standpoint of the infected serviceman. For example, if infected by mosquitoes which inject a definite number of microfilaria, it is impossible to ever have more than that number of adult worms, regardless of how long the individual lives. In other words, the adult worms are unable

to reproduce themselves in the body and thus do not continue to accumulate.

The pathological and clinical manifestations seen in the serviceman are undoubtedly stirred up by the irritation of developing worms in the lymph glands. Three predominate findings are noted in the early infections, namely, lymphangitis, lymphedema and lymphadenopathy. The lymphangitis is always retrograde, usually starting in the axilla, elbows or groins and then slowly descends. The extremes vary from the length of the extremities to a few centimeters. Although somewhat painful, it does not compare to the pain encountered in a streptococcic lymphangitis. Systemic toxic manifestations are practically absent. There may be a temperature of 101° to 102°F., which usually lasts about 48-72 hours. There still exists considerable controversy as to whether this lymphangitis, which is probably an allergic manifestation producing a lymph blockage, is associated, primarily or secondarily, with a streptococcus. The supporting evidence is not good for this theory, and the administration of sulfonamides or penicillin has no apparent effect on the lymphangitis. Lymphedema usually tends to be localized, although occasionally it is generalized, involving the entire arm or leg. An important point is that thus far it has been temporary, as no permanent swellings indicative of elephantiasis have been noted at this station in several hundreds of men over a period of eight months.

The picture of filariasis as it exists in Marine personnel from the Pacific can best be furnished from an analysis of some of the cases seen to date. In a large series of cases, 96% were stationed in the Samoan group of islands, and only 4% have contracted the infection elsewhere, Guadalcanal or Bougainville. This has occurred in spite of the fact that surveys on natives in the two general areas show intensities of infection that are not too different. The reasons for this disparity may be accounted for by the living habits of the natives, opportunities for contact, vectors and the periodic appearance of the microfilaria. In Samoa, the average length of stay was 12.5 months, with a variation of 1 month to 27 months. The incubation period, dated from the time of the first possible exposure to the appearance of initial symptoms, averaged 9 months. When seen in the States there was little differentiation in the degree of involvement in the men who were in the endemic areas a few months as compared to those who were present two or more years. The anatomical involvements as lymphedema, lymphadenopathy and lymphangitis are noted in the sites and frequency as follows: Neck—2%, upper extremities—30%, lower extremities—9%, scrotum—1%, cords—15%, and testes—18%. It was interesting to note that the right upper extremity was involved initially in 54% of the instances, while the left was only involved in 37% of the instances. For the lower extremities, the right exceeded the left, but the difference was not so marked; more use of the right arm might provide an explanation. However, for the genitalia the opposite was true; 33% on the right and 55% on the left.

From the picture of filariasis as it now exists in the serviceman, it is quite apparent that the current manifestations are extremely mild. The laboratory examination of the infected individual shows no abnormalities of the blood with the exception of an occasional instance of eosinophilia, which is rarely sustained. Thus far, three patients have come to our attention who have had high total white blood cell counts and unusually high eosinophile counts. Other than a chronic cough, there were no symptoms. Temperatures were within the normal range. The clinical histories and maximum blood counts were as follows:

Patient B., L. Cough 5 months, WBC, 36,150, Eosinophiles 72%;

Patient L., L. Cough 4 months, WBC, 14,600, Eosinophiles 48%;

Patient, R., A. Cough 16 months, WBC, 16,550, Eosinophiles 45%.

No microfilaria have been seen in the blood of any man on our station. There are a few verified instances when they have been observed in Army personnel following the concentration of large amounts of blood. Removal of enlarged lymph nodes for biopsy has been done and adult worms were revealed on histological examination, and in some instances microfilaria were detected *in utero*. In others, the adult worm was undergoing degeneration or calcification. This is not a recommended procedure from a therapeutic standpoint. However, it has provided valuable information in that it is now known that the body is gradually eliminating the infection. As yet there is no chemotherapeutic agent that is known to destroy the infection. H. W. Brown (6) has stated that a pentavalent antimony compound, known as anthiomaline, will decrease the microfilaria count in Virgin Island natives, a finding of doubtful importance unless it can be demonstrated that the

adult worm is also destroyed. The use of heat, cold, X-ray, non-specific proteins, etc., has not been shown to be beneficial.

There is a very prominent psychic element in the picture, as could be expected with an infection that involved the genitalia plus the reaction after seeing the grotesque deformities in the natives with elephantiasis. Men are concerned about sterility and the possibility that continued assaults on the lymphatic system will leave a permanent lymphedema. We now have had many hundreds of men under constant observation for several months. Upon returning from overseas, many want to get married, do, and have already started families. In 249 married men questioned there were 45 instances where the wife was pregnant. This disposes of the sterility factor. As far as the possibility of servicemen developing elephantiasis is concerned, this is an extremely unlikely possibility. Elephantiasis, in natives, usually appears late in life and rarely involves more than 5% of the population, even in the most highly endemic areas. They have been continuously exposed to the bites of infected mosquitoes from the moment of their birth until this complication appears. Elephantiasis and filariasis are not synonymous terms, but elephantiasis is a complication that arises after many years where the assaults on the tissues have been constant throughout that period. The differences in the intensity of infection are quite plain when one examines the blood, as the natives show 60% or more having high concentrations of microfilaria in the blood, whereas, in the serviceman it is a very unusual find to observe it in any individual. The secondary cases of filariasis which appeared in Charleston, South Carolina, a focus of infection which has now disappeared, were rarely associated with elephantiasis, because, like the serviceman, they were also very lightly infected. Brigadier N. H. Fairley (7), in Australia, reports that in North Queensland, 10% of the white population in 1920 had circulating microfilaria. This focus has also disappeared and no case of elephantiasis ever developed.

Of interest to all physicians and public health officials is the question as to whether filariasis is likely to gain a foothold in this country from the infected cases. It is the opinion of the author that this likelihood is extremely remote if not *nil*. In the first place, circulating microfilaria, if present, have been observed so infrequently and so few in number that transmission by mosquitoes would be impossible. Actually, no one has been able to infect artificially any local mosquitoes. Secondly, climatic conditions in this country, with the exception of a coastal strip in Southeastern United States, is not conducive to the maintenance of the disease. Finally, the infection as manifested in diagnosed cases is becoming more and more mild. Therefore, the future as regards the secondary transmission of filariasis in the United States should hold no cause for alarm.

This brings us to a consideration of the type of care best suited to these men returning with questionable or positive clinical symptoms. The term "questionable" is used because the clinical picture is the only means of establishing the diagnosis. It has been our policy to interview each man as he returns and explain to him very carefully the status of his infection. The following points are brought out. Since the microfilaria must continue their development in the mosquito, there is no possibility that the adult worms in the human body can reproduce themselves and thus they are not accumulating. Also the body is constantly destroying them. Therefore, the end result almost certainly has to be a good one and another year or two should see the disappearance of practically all symptoms in most cases referable to this disease. The man is then placed on a full duty status in a post where the terms "recreation" and "convalescent" have been purposely omitted, as there is no better way of convincing a man that he belongs to a misfit group. He is given a series of gradually increasing physical exertion exercises in combination with a program encouraging outdoor recreation. There is an immediate response, especially on the mental side, and it can now be stated that the majority of all men are not apprehensive about their infection. They do continue to have occasional flare-ups of edema, lymphangitis, and muscular soreness, but this persists only for a few days, and the following attacks become less and less frequent. Actually, it has been necessary to hospitalize only one man in 800 for filariasis, and the average stay has been five days. Less than 5% report to sick call with objective findings and 12% with subjective symptoms. There is one factor which remains to be assessed, namely, the time factor, but all the accumulated evidence to date indicates that filariasis will not result in permanent disability as has been feared and postulated by many. The abuse of rest is very apparent during the convalescent period from this disease, as it is being observed in many other infections or disabilities, and certainly

any man with filariasis needs very little encouragement to make him a permanent mental and physical cripple. Without curative drugs the program of care has been directed toward mental and physical rehabilitation. A daily program of directed military and recreational activities has been instituted. Some of these activities are very strenuous and serve as objective demonstrations that filariasis is not a crippling disease. After several months' observation of several hundreds of cases, it now seems that the problem of filariasis is not a serious one and that the vast majority of individuals now infected will shortly experience their final difficulty. In the interim between *inception of symptoms and recovery, the most* important procedure is the prevention of "hospitalitis" by a sane and interesting program of mental and physical activity.

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CLINICAL AND PUBLIC HEALTH ASPECTS OF MALARIA IN THE UNITED STATES FROM AN HISTORICAL PERSPECTIVE[1]

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INTRODUCTION

From the time of early Colonial history until the present day malaria has constituted a disease of more or less major importance in the United States, with the development of extensive hyperendemic territory in the warmer Southern States and a much larger area of milder endemicity in the contiguous regions to the north and west. At times the disease has broken out in epidemic form and has constituted a real menace to human existence. Unfortunately the amount of malaria in the United States up to the last twenty five years cannot be accurately assessed, first of all because accurate records of morbidity and mortality were not kept in many of the malarious areas and second because in the earlier decades malaria was not differentiated from other diseases producing remittent or intermittent fevers, or from typhoid fever. Nevertheless, there is considerable intrinsic evidence in the early medical history of the United States which indicates the general trends of the disease.

THE EARLY HISTORY OF MALARIA IN THE UNITED STATES

There is no indication that malaria existed in the United States or elsewhere in North America prior to the advent of the European conquerors and settlers (1). Certainly there was abundant breeding of malaria-transmitting mosquitoes, so that propagation of the disease would not have been prevented for lack of appropriate mosquitoes. This is concretely illustrated by the ease with which the disease developed and spread just as soon as a sufficient number of human carriers were available.

It is entirely probable that some of the British explorers and settlers suffered from malaria, since the disease was at that time endemic in the Fen counties, Essex, Kent, Surrey and Summersetshire, and undoubtedly some of the Spanish and French explorers were infected (2). Yet there is no evidence that malaria developed to alarming proportions until the importation of the negro into the Southeastern States. This was particularly noted in the Carolina tidewater area when rice cultivation was undertaken on a relatively extensive scale (3), as well as in the rich alluvial country of the Gulf Coast when sugar-cane production was started. Here forests were cut, canals were dug and rice fields or cane lands were inundated, providing an unusual opportunity for *Anopheles quadrimaculatus* to breed and thus serve as transmitter of the malaria parasites from infected negroes to their uninfected associates and to the white plantation population. Moreover, the congested unsanitary conditions under which the negroes existed on the slave ships which brought them to this country must have activated malaria and other disease processes to which they had been exposed in Africa, so that both the human seed beds and the new environment were favorable for the rapid development of these infections. In addition, the negro was more or less tolerant to the strains of the malaria parasites to which he had been exposed in Africa but the white man, who acquired malaria following infection of susceptible American mosquitoes, was completely non-immune. Thus, the disease became highly endemic and by the time of the American Revolution it had spread along the Atlantic coast from Georgia to Pennsylvania.

Even before the Federal Government had been established pioneers began a westward trek for new land and by the beginning of the nineteenth century this migration had measurably increased. In this way malaria was carried up the river valleys of the Atlantic coast, across the Appalachian divide into Western New York and Pennsylvania and into the Ohio river valley. From the more southern Atlantic Coast States it was spread into Kentucky, Tennessee, Alabama, Mississippi, Arkansas and northern Louisiana. Meanwhile, French settlers brought malaria di-

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rectly to Mobile, New Orleans and Natchez, while Spanish immigrants from Mexico and other countries to the south introduced the disease into Texas and the Gulf Coast.

Little by little, as new land came into cultivation and an adequate human population was provided, malaria became firmly established from the tip of Florida to the New England States, and westward practically without interruption to Arizona and New Mexico, eastern Colorado, Nebraska, southeastern South Dakota, and all of Minnesota and Wisconsin except for the northernmost counties of these two states. With the settling of the Lake Erie shores of the Canadian Province of Ontario malaria developed a foothold in that region. Moreover, the early overland immigrants to the West coast carried the disease with them, where it took root in the lower Columbia river valley and in the Sacramento-San Joaquin valleys in California (2). By 1850 practically the entire United States constituted one vast expanse of malarious country, except for Maine, the northern portions of Wisconsin and Minnesota, the Appalachian highlands, the cool arid northwest plains, the Rocky Mountain area, the western desert and the Sierra ranges. Much of this territory was only mildly endemic, with epidemic outbreaks during the warm summer months; but in an extensive southeastern triangle from Baltimore to central Florida and westward to the state of Mississippi, as well as in the Ohio-Mississippi drainage and in eastern Texas, malaria was hyperendemic.

MALARIA DURING THE MIDDLE PART OF THE NINETEENTH CENTURY

Viewing the malarious regions of 1855–1860 from south to north, the medical historians of the day (2) found the disease widespread from the Rio Grande to Florida. On the Western plains there were only a few scattered foci. In Texas it was particularly prevalent in the Colorado and Brazos river valleys. It was widely disseminated among the Indians in New Mexico and was a serious medical problem at Forts Bayard and Union which were located more than a mile above sea level. The disease was hyperendemic between the Mississippi and Sabine rivers in Louisiana, and extended with equal intensity into Arkansas, Missouri and the Indian territory. Army forces at Fort Sill felt its effects, but more particularly at Fort Gibson, which was designated the "charnel-house of the Army". On the Gulf coast east of the Mississippi river and extending almost to Pensacola, Florida malaria was relatively light in its distribution and the "piney woods" and low coastal hills were reputed for their salubrious climate. On the other hand, from Natchez to Memphis "Yazoo swamp fever" was hyperendemic. In the vicinity of Mobile Bay and in the valleys of the Alabama and Black Warrior rivers, as well as in the marshy plains of Huntsville near the Tennessee border, the disease was indeed serious. In Florida the regions of hyperendemicity included Escambia and Gadsden counties, Tampa Bay and Fort Meade. Although the situation was considerably better on the east coast of Florida from St. Augustine south, the plains of central Florida and the swampy coast of southeastern Georgia were highly malarious.

Inland from the "deep South" there was widespread prevalence up the Mississippi and Ohio river valleys. This condition applied to western Tennessee, to the Ohio river drainage, the "barrens" of Kentucky, and to the adjacent portions of Missouri, Illinois, Indiana and Ohio. An appreciable malariousness existed throughout northern Missouri, Iowa and the southern half of Minnesota and Wisconsin. At Forts Snelling and Ripley near Minneapolis, 15 per cent of the troops were ill with malaria, but in the Dakota Territory and Montana the incidence was only 5 to 6 per cent. The disease was relatively prevalent in southern Michigan, the peninsular part of Ontario, throughout central and northern Ohio, western Pennsylvania and the areas of New York adjacent to the Great Lakes and the St. Lawrence river. On the other hand, the counties of New York in the Finger Lakes district (Onondega, Tompkins, Seneca, Ontario and Oneida) were freer of malaria than they had been some years earlier. Malaria was still common in the Hudson river valley and along Long Island Sound, and had increased in the mountain valleys of New York and Pennsylvania, but in New Jersey and New England the situation was somewhat improved.

In the western states and territories malaria was endemic in a few valleys in Wyoming, Colorado and Utah, especially in Indian settlements, and was a serious menace in Arizona. It was believed not to be indigenous in Washington and Oregon; however, it was firmly established in the Sacramento and San Joaquin valleys of California, although it did not occur on the coast from Monterrey to San Diego.

In general, it may be concluded that from relatively early colonial days malaria gradually increased in intensity in the foci where it first became established along the Carolina and Gulf coasts, then extended coastally and inland and finally became endemic throughout practically all of the potential malaria territory within the confines between the Atlantic and Pacific, the Gulf and the Great Lakes. By about 1855 it had reached a peak and during the next quinquennium was declining. This view is confirmed by the vital statistics of the Surgeon-General's Office, U. S. Army (4). Between 1840 and 1854 the malaria mortality rate of troops per 1,000 cases of malaria was 108.02, but during the period between 1855 and 1859 this rate was reduced to 61.08.

Ackerknecht's recently published monographic account, entitled "Malaria in the Upper Mississippi Valley (1790–1900)" (5) indicates that the disease in Illinois was highly prevalent from 1760 until 1870, when it began to decline and then dropped precipitately between 1880 and 1890. In Missouri the high incidence plateau existed between 1820 and 1870, but marked decrease did not begin until 1900. In Iowa and Wisconsin the high incidence rates prevailed between 1830 and 1870, with a notable decline between 1890 and 1900. Minnesota was rather highly malarious for the single decade between 1860 and 1870. In all of these five states in the Upper Mississippi basin the peak mortality occurred about 1860 (Illinois with 37, Missouri with 112, Iowa with 79, Wisconsin with 57 and Minnesota with 26 malaria deaths per 100,000 population).

This same writer refers to certain epidemiologically distinct types of malaria in the United States, based on their characteristic behavior. These include 1) the New England type, embracing New England and possibly parts of New York State and Virginia; 2) the Midwestern type, which prevailed in Ohio, Indiana, Michigan, Illinois, Missouri, Iowa, Wisconsin and Minnesota; 3) the Southern type, "comprising those states where malaria is endemic to this day and where considerable decrease started only during this century", and possibly other types, as a New Jersey-Maryland type. No mention is made of the epidemiological varieties of malaria in Oklahoma, Texas, the Southwest or the inland valleys of California. Furthermore, southeastern Missouri and the southern tip of Illinois belong to the "Southern type", if such a distinction is actually justified.

MALARIA AND THE CIVIL WAR

With the advent of the War between the States and the quartering of relatively non-immune (because previously unexposed) Federal forces in the malarious South, malaria in the U. S. Army again increased. Considering the Army as a whole, the cases per 1,000 mean strength were as follows: 390.7 for 1862, 428.3 for 1863, 535.8 for 1864, and 496.4 for 1865. However, in the departments in the most malarious areas there was a notable difference among white troops from those in mildly endemic areas. This is illustrated in the averages for the period 1862–1865 as follows: Dept. of North Carolina, 1087.1 cases and 3.6 deaths per 1,000 mean strength; Dept. of the Tennessee, 848.1 and 5.9; Dept. of the Gulf, 803.4 and 4.8; Dept. of the Northwest 201.0 and 0.6; Pacific Region, 197.4 and 0.3, and Dept. of the East, 186.3 and 0.2. During the latter part of this period the negro troops in the U. S. Army quartered in the South were nearly twice as heavily infected as the white troops, but the ratio of deaths to cases was only 5 among the negroes compared with 9 in the whites. On the other hand, death from typhoid fever among negro troops greatly exceeded that of the whites (4).

In the Department of the Gulf, principally centered around New Orleans, quotidian malaria (estivo-autumnal?) far exceeded tertian malaria during the year July, 1861 through June, 1862 but during the next three fiscal years tertian malaria was somewhat more prevalent. Quartan malaria was at first incidental but by the fourth year had become approximately six per cent of the total cases.

The high malaria morbidity of Federal troops stationed in the vicinity of New Orleans in the years immediately following the war remained high. All four forts near the city were hotbeds of the disease, while at Baton Rouge, in 1868 there were 1033 cases of malaria in a mean strength of 227 (4).

THE PERIOD OF RECONSTRUCTION

The first two decades after the Civil War showed no abatement of malaria among the civilian population in the South. The Armies of the Confederacy had drawn the effective white manpower from the plantations, while most of the emancipated negro slaves were making poor use of their newly acquired freedom. Thus the fertile bottom lands of the Atlantic seaboard, of the Gulf coast

and the Mississippi delta region from New Orleans to Cairo remained for the most part uncultivated and undrained and provided extensive breeding grounds for malaria-transmitting mosquitoes. Moreover, the poorly nourished human population offered an increased opportunity for the disease to gain the ascendency.

The following data are suggestive of the drain which malaria continued to make on the economy of the United States during the last three decades of the nineteenth century. In 1874 at Cairo, Illinois twenty per cent of all hospitalized cases had malaria (6). In 1881 the malaria death rate in Shreveport was 0.43 per cent of the total population; in Vicksburg, 0.32; in Baton Rouge, 0.17; in New Orleans, 0.1, and in Natchez, 0.06. All of these are today considered to be tropical death rates for the disease. During this period malaria continued to be prevalent along the Missouri river bottoms, was a frequent source of illness in southeastern Kansas (6), was common in counties of Iowa and Illinois bordering on the Missouri river and in the Illinois and Wabash river drainages. It was highly endemic in parts of Northern Illinois, Indiana, Ohio and Michigan bordering on Lakes Michigan and Erie, and cases were commonly seen on the wards of the Johns Hopkins Hospital, the Philadelphia General Hospital and the Massachusetts General Hospital during this period. In the 1880's Ontario was still highly malarious along the shores of Lake Erie and Lake Ontario (6). The disease was even moderately prevalent on Manhattan Island (6).

DISCOVERY OF THE ETIOLOGY AND TRANSMISSION OF MALARIA

Although Laveran had discovered the etiological agents of malaria in 1880, for a decade his findings failed to find recognition among American physicians. But during the 1890's Dock (7, 8), Thayer (9, 10, 11), Welch (12), MacCallum (13, 14), Craig (15, 16, 17), and others made substantial contributions to the morphology and life cycle of the malaria parasites and to the relationship of the etiological agents to the production of the disease. These observations, supplementing the basic investigations of Ross and Italian students of malaria, placed the etiology and epidemiology on a sound foundation, yet the diagnosis of the disease by demonstration of the plasmodia in blood films was not enthusiastically undertaken except in a few medical centers as Boston, Philadelphia, Baltimore and New Orleans. In the average case malaria was still diagnosed by clinical methods alone and was frequently confounded with typhoid fever, while diagnostic ignorance was masked under the convenient term "typho-malaria". This situation existed in the U. S. Army during the Spanish-American War (1898–1899). Colonel Charles F. Craig has related to the writer that at Camp Chickamauga, Georgia, at an elevation well above the usual malarious zone, many fevers were diagnosed as malaria by enthusiastic young Army surgeons and the patients given courses of quinine, whereas most of them were suffering from typhoid fever and some actually died of the disease while under quinine therapy.

Even with the advances which have gradually developed in the understanding of malaria in the United States between 1900 and 1945 a considerable proportion of physicians in endemic areas, when left to their own initiative, are content to rely solely on clinical diagnosis, while many physicians outside present-day malarious areas are not even conscious of the possibility of malaria in their patients and have no idea how specific diagnosis is accomplished.

DECLINE IN MALARIOUSNESS

During the first two decades of the present century there was cumulative evidence, as Maxcy (18) phrased it, "that the northern border of the 'malaria belt' has been retreating". He demonstrated for the first time on the basis of malaria mortality data by counties that this was occurring in Missouri, Indiana, Kentucky and Virginia. The death rate per 100,000 population in Missouri was 17.0 in 1910 and 4.0 in 1920; in Indiana it was 10.0 in 1900 and 0.7 in 1920; in Kentucky it was 10.0 in 1910 and 1.0 in 1920, and in Virginia it was 3.0 in 1915 and 1.0 in 1920. Moreover, Barber (6) found it to be greatly reduced in southern Illinois, and Vaughan (18) compared the malaria mortality rate of 20.0 per 100,000 in Michigan during the 1880's to 0.1 or lower after 1915. Similar favorable declines were reflected in the malaria cases and deaths in the Massachusetts General Hospital, the Johns Hopkins Hospital and the Charity Hospital in New Orleans. What conditions or factors were responsible for this improvement?

From the time of its introduction from Europe and Africa it was observed that malaria increased wherever opportunity was provided for the abundant breeding of mosquitoes around human habitations, in other words, wherever man-made malaria

was possible. This commonly occurred when forests were cut and virgin sod was turned over for the cultivation of crops, and in the more arid prairies of Kansas and Nebraska where artificial ponds were constructed for watering livestock (6). Only when land was brought under total cultivation and was scientifically drained, or when artificial ponds were replaced with windmills was the mosquito hazard minimized and malaria proportionately reduced. Examples are abundant and include the tobacco-growing areas of the Connecticut river valley, the vineyard lands of central New York State, the swampy suburban area east of Cleveland, Ohio, the cornfields of Indiana, Illinois, Iowa and Northern Missouri and the wheat lands of Kansas, Nebraska and the eastern part of the Dakotas.

Added to the above breeding grounds were those occasioned by the building of railroads with their "borrow pits" to fill in grades, and in the natural lowlands of innumerable valleys which flooded at times of high water and provided many residual pools favorable for anopheline breeding after the overflow had receded. Right-of-way breeding along railroads occurred all over the country and similarly the undrained water left after spring floods constituted widespread spawning grounds for the mosquitoes. The gradual filling in or draining of these breeding places reduced the liability of transmission.

Probably equally important was the reduction in the price of quinine, from $4.50 per ounce in the 1880's to 25 cents in 1913 (6). This brought it within the reach of the common people who were most frequently exposed to infected mosquitoes. The family ritual of taking quinine every day with coffee appreciably decreased clinical malaria and greatly lowered the malaria death rate.

Nevertheless in 1920 malaria was by no means a disease of historic interest only. It is true that hyperendemic belts had been limited primarily to a southeastern triangle from the coast of North Carolina to Central Florida and west to Central Alabama; to the Mississippi delta from Cairo to Natchez; to the counties of Arkansas, Louisiana, Oklahoma and Texas where these states join, and to the lower Rio Grande; but at least 90 per cent of the South was still malarious. However, except in scattered surveys accurate morbidity data were essentially lacking and several of the highly malarious states (Alabama, Arkansas, Georgia, Oklahoma and Texas) were as yet not in the U. S. registration area, so that even dependable mortality statistics were not available.

ANNUAL MORTALITY DATA FOR MALARIA

I became interested in malaria in the South in 1928 when I came to occupy the newly created professorship of parasitology in the Tulane University School of Medicine, the first medical school in the United States to recognize the need for training undergraduate medical students in the practical aspects of human parasitology. Most of the Tulane medical graduates were practitioners in malarious communities in the southern United States and in order to understand their problem it was important to obtain whatever concrete information there was available on malaria for the entire area. Beginning with the year 1930 (20) and continuing each year to the present time (21–33) inquiries have been directed, under the auspices of the National Malaria Society, to state bureaus of vital statistics for the purpose of obtaining yearly malaria mortality data for the state as a whole and for each of its political subdivisions. Beginning with the data for 1933 (25) Ohio, Indiana, Illinois, Kansas and California were added to the territory surveyed and by 1939 (29) all of the forty-eight States and the District of Columbia were providing information annually.

Mortality data were chosen as a basis for evaluating the status and trends of malaria because the causes of death are required to be certified in each of the States and, although they are certainly not invariably reliable in individual cases, *en masse* they are usually statistically significant. The year 1929 was selected as the starting point for the accumulated data, since this was the first year in which all of the states in the highly malarious territory except Texas were in the U. S. Registration Area. However, total malaria deaths per year have been accumulated in the record for years antedating 1929 as follows: Alabama, from 1925; Arkansas, from 1927; Florida, from 1919; Georgia, from 1921; Kentucky, from 1915; Louisiana, from 1918; Mississippi, from 1919; Missouri, from 1915; North Carolina, from 1915; Oklahoma, from 1928; South Carolina, from 1916; Tennessee, from 1917, and Virginia, from 1915; and, in addition, California, from 1915; Illinois, from 1918, Indiana and Ohio, from 1915 (U. S. Bureau of the Census, Division of Vital Statistics). Thus, in several of these states a malaria mortality curve is available for inspection over a twenty-five to thirty-year period.

Attempts have been made on several occasions (23, 25, 31, 32, 33) to obtain malaria morbidity data from the state bureaus but even in the more malarious states information has been practically valueless, partly because of self-treatment by patients, partly as a result of the widespread practice by physicians of making a presumptive clinical diagnosis without microscopic examination of blood films and partly due to carelessness of physicians in keeping records. Frequently in certain highly malarious areas the number of certified malaria deaths has been and still is higher than the recorded cases of malaria. This means that even today in certain localities there is no official record of malaria until a patient dies of the disease.

During the Civil War and the Spanish-American War troops quartered in camps in the South had a high ratio of malaria deaths to cases, but during World War I, with early accurate blood-film diagnosis and thorough treatment, the Army forces in the same malarious areas experienced the favorable ratio of one death for each 415 cases (34). This has provided the only actuarial basis for the period on which to compute the probable number of malaria cases occurring within the area (35).

MALARIA MORTALITY TRENDS SINCE 1915

A study of the mortality rates by states as they have become available since 1915 indicates that there have been periodic peaks and depressions in the rate curves, apparently associated with an increase and decrease in malariousness. Although there has been some variation in the peak years in different states, the cycle has been repeated about every five to seven years, for example between 1914–1918, 1920–1922, 1927–1929, and 1933–1935 (see fig. 1). Another peak was anticipated between 1938 and 1941 but with the exception of a very slight increase in the rates for Alabama, Louisiana, Kentucky and Tennessee in 1938, for North Carolina in 1940 and for Texas in 1941 there has been no change in the unusually favorable decline in malaria deaths since 1935.

No satisfactory explanation for the cyclic changes in the malaria mortality rate curve has been advanced. It does not fit in with solar cycles which have been cited as governing biological trends about every eleven years, and no one has produced a plausible meteorological explanation, although the amount of precipitation may have had some causal connection. However, with few exceptions the mortality rates for the 1933–1935 peak were higher than the two previous peaks (25) and this situation appears to have been directly related to the severe economic depression at the beginning of that period. Actually the upward trend was partially predictable in the 1932 returns (23), but it developed more rapidly than had previous cyclic increases.

The high mortality of the early depression years (1933–1935) (23, 24, 25) is attributable to a number of factors, including the low market value of cotton, rice, sugar and tobacco, with resultant reduction in cultivated land. On the one hand this permitted increased mosquito breeding, as during the Reconstruction Period from 1865 to 1880, and on the other hand reduced the earnings of tenant farmers and share croppers below a subsistence level, while hired labor was for the most part entirely on relief. Quiescent cases of malaria became active, many new cases developed and there was no money to buy anti-mosquito sprays or even quinine. For a time anti-malarial drainage projects were undertaken to get the unemployed on relief as quickly as possible but without consulting Federal or state sanitary engineers as to the effect of ditch digging on the control or spread of the disease. In the opinion of qualified state public health officers this emergency measure actually increased malaria in some highly malarious areas, as in South Carolina, Florida and Mississippi, and carried it into adjacent territory. However, just as soon as Federal funds became available through the U. S. Public Health Service, an intensive sanitary campaign was undertaken in cooperation with state departments of health (27) to bring about malaria control in the following ways: 1) larvicidal control through extensive scientific drainage and through spraying of *Anopheles*-breeding areas; 2) establishment of central and regional laboratories in every malarious state for training technicians in the accurate diagnosis of malaria parasites; 3) encouragement of physicians throughout these states to send in to the state laboratories blood films of all suspected cases of malaria; 4) introduction of atabrine to supplement quinine and in later years in a considerable degree to supplant quinine in the treatment of malaria, and 5) not least in the armamentarium, development of a better state of nutrition among the poorer classes, who were most frequently exposed and most commonly have felt the direct effects of malaria through the sapping of their vitality. Added to the above measures have been the use of anti-mosquito sprays on an extended scale and increased and better screening of homes, particularly as demonstration projects in

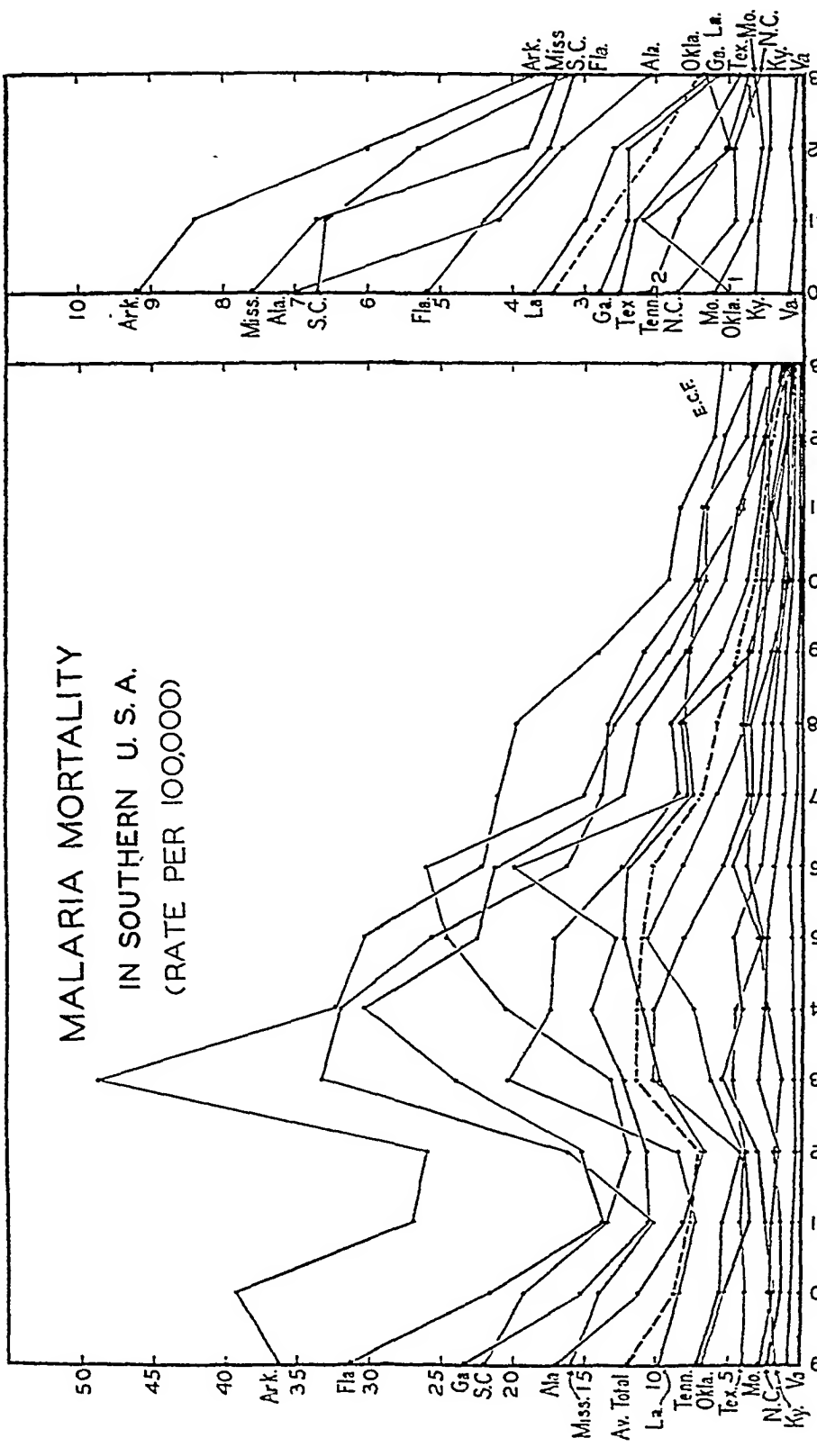

FIG. 1. Chart showing the year-by-year malaria mortality rate per 100,000 population for each of the fourteen malarious states in the southern United States, 1929–1943, together with the average rate each year for these states. On the right the rate scale is enlarged for the purpose of clarification.

certain areas. Moreover, the Tennessee Valley Authority, through its Health Department, has served as a large-scale, scientific field laboratory, to test and put into practical operation certain aspects of naturalistic control of mosquito breeding, as well as antilarval spraying and treatment of cases. These measures which have just been outlined have in no small measure been responsible for the rapid decline in the malaria death rate in the South and for the general improvement in the malaria situation in adjacent territory.

CHANGES IN THE MALARIA PICTURE IN THE LAST TWENTY-FIVE YEARS

In 1936 Dauer and Faust (24) remarked: "It is extremely interesting that a map published by Maxcy (18) in the Public Health Reports in 1923, showing the average mortality by counties for 1919 to 1921 inclusive, covering this same area," i.e., the southern United States, "is almost a counterpart of the map we have prepared. Not only are the outlines of the endemic areas approximately the same, but the mortality rates are quite similar except in Virginia and North Carolina" (where they had notably decreased). The map referred to by Dauer and Faust (24) was for the four-year period 1929–1933. Later I prepared a map for the entire United States showing the average mortality rate for the ten-year period 1929–1938 (36). This map (fig. 2) showed the same areas of hyperendemicity as well as the contiguous regions of milder endemicity extending to the northeast, north, southwest and with foci on the Pacific coast. The situation had vastly improved since 1850–1860 but still constituted an extensive public health problem which required attention.

By 1940, however, the status had become notably better (30) (see fig. 3). Although 34 per cent of the counties in the southeastern United States and the adjacent eastern plains area of Texas each had one or more malaria deaths for the year 1940, the mortality rate exceeded 25 per 100,000 population in only twenty-eight counties (six in Alabama, two in Arkansas, five in Florida, five in Georgia, three in Mississippi, five in South Carolina and two in Texas), and of these only one (Dixie Co., Florida) had a rate in excess of 50. By 1941 (31) the situation was greatly improved in Alabama, better in Georgia, but with more counties having a rate of 25 or higher in Florida (six), South Carolina (seven) and Texas (four), and one each in Louisiana and Tennessee. The total number of counties in the South with a rate in excess of 25 was one more than in 1940 but the total reported deaths for the fourteen most malarious states was 1113 (average rate, 2.73) instead of 1346 (average rate, 3.4) for 1940. By 1942 (32) only four counties had a mortality rate of 25 or more and the average rate for the fourteen southeastern states and Texas was reduced to 2.02, with 692 deaths. However, there was an appreciable average rate increase during the year in Kentucky (from 0.42 to 0.46), in Missouri (from 0.53 to 0.66) and in Oklahoma (from 0.98 to 1.28).

The most recently collected data (e.g., for 1943) (33) indicate an overall continued progress in the favorable condition. There were seven counties (one in Arkansas, two in Florida, one in Mississippi, one in Missouri, one in North Carolina and one in Texas) which had a mortality rate in excess of 25. None of these counties was in the 1942 list and only one in the 1941 list. This indicates considerable annual fluctuation in the rates of counties in hyperendemic territory, so that the date by counties must be regarded as significant only when a high or low rate is maintained over a period of years. However, for 1943 the average rate for the fourteen Southern States was reduced to 1.46, with 576 deaths, showing a continued general decline in the death rate. In a decade the malaria death rate in these states had declined from 12.5 (1934) to 1.46 (1943), or 88.3 per cent.

Within the same period of time the average malaria death rate for the entire United States dropped from 3.43 (1934) to 0.47 (1943), a reduction of 86.3 per cent. Thus, the decrease in deaths throughout the country has paralleled that in the more intensely malarious South. During this period the percentage of total malaria deaths for the South, compared with the entire United States fluctuated considerably. In 1934 it was 96.7; in 1941, 80.0; in 1942, 94.3, and in 1943, 92.6.

MALARIA MORBIDITY DURING THE PAST DECADE

It has already been stated that malaria morbidity rates in the United States have been unreliable for statistical purposes and the reasons for their unreliability have been given. But since 1941, there is one important reason for believing that a significant reduction in malaria cases should have occurred, namely, the unusual precautions taken by the U. S. Army inside military camps (37) and similar preventive measures of the Division of Malaria Control in War Areas of the U. S. Public Health Service immediately around military reservations (38), to prevent malaria from developing

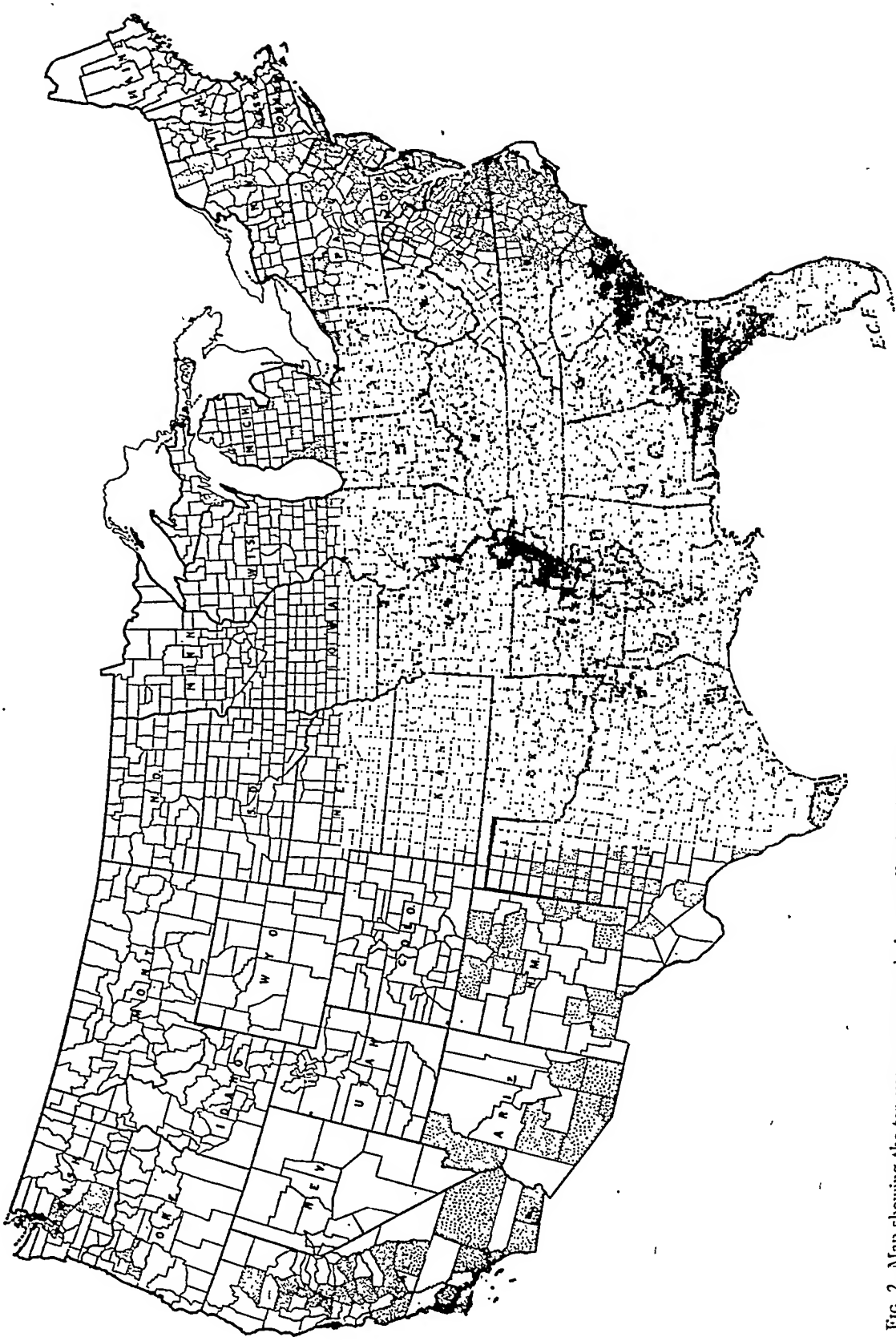

FIG. 2. Map showing the ten-year average malaria mortality for the United States by counties for the period 1929–1938. Solid black indicates an average rate of 50 or more per 100,000 population; heavy stippling, a rate of 25 to 49.9; light stippling, less than 25; white areas, no reported deaths for the decade. (After Faust, courtesy Am. Assn. for Adv. Sci.)

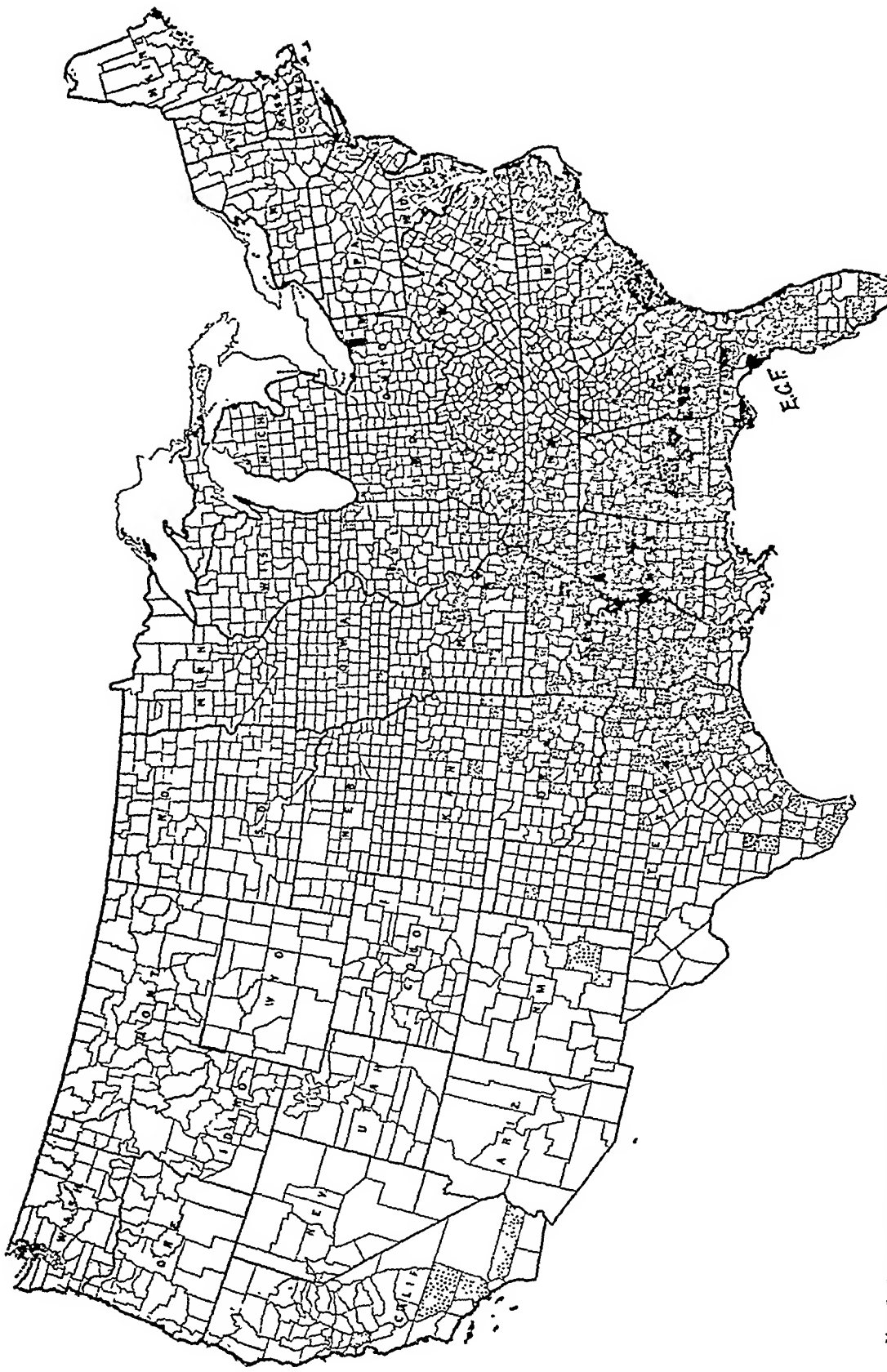

FIG. 3. Map showing malaria mortality rates for the United States for the year 1940. Legend similar to that of fig. 2. (After Faust, courtesy National Malaria Society.)

in the military and civilian populations of these areas. The significant improvement in malaria case rates of military forces within the Continental United States has been emphasized by Simmons (37), who has published charts comparing malaria morbidity in the troops within the country in World War I and World War II. This provides concrete evidence of the effect of the control measures instituted in and around encampments. Actual figures for World War II cannot yet be released but through the courtesy of the Division of Preventive Medicine of the Surgeon-General's Office I am permitted to include in this paper a recently prepared chart showing the amount of malaria per 1000 men per year in the Army forces who have not been overseas, the amount in the overseas forces and that in the Army as a whole (see fig. 4). This chart supplements the one published by Simmons (l.c.) in showing how effective malaria control measures have become.

Within the decade there has been evidence of increased interest in the amount of malaria in the United States. In the South there have been state-wide malaria surveys in North Carolina and Mississippi (33). These have indicated that the disease is still widespread and at least in Mississippi is endemic in every county of the state. The information is important because it provides an index of potential widespread danger at a time when greatly reduced malaria mortality might suggest that precautions could be relaxed. As I stated in 1939 (27) so today I may reaffirm, "an unduly high percentage of the population in the South lives in malarious territory."

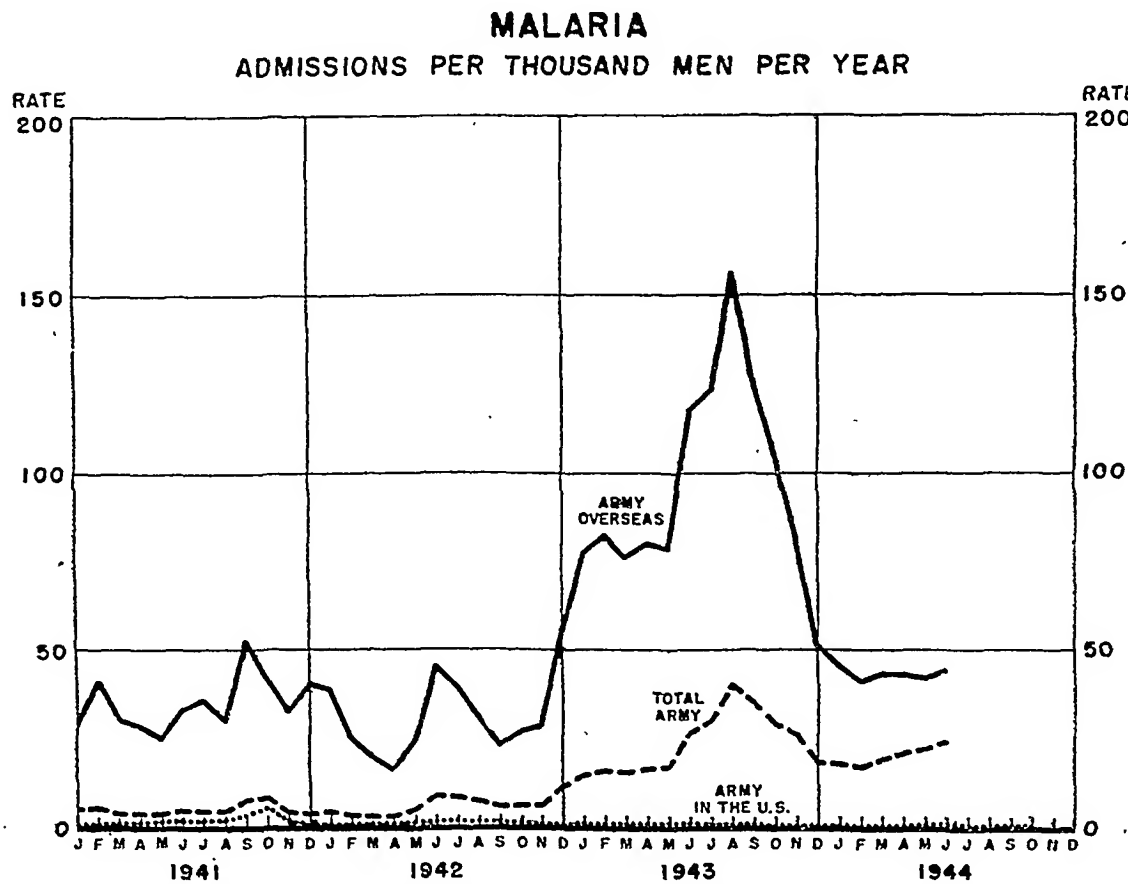

FIG. 4. Chart showing malaria morbidity in the U. S. Army 1) in the Continental U. S., 2) overseas and 3) total Army, January 1, 1941 through June 30, 1944. (Prepared by, and permission for publication generously granted by the Division of Preventive Medicine, Office of the Surgeon General, U. S. Army.)

In mildly malarious areas and those in which malaria is no longer endemic, as in most of New England and the more northern prairie and Rocky Mountain states, epidemiologists and vital statisticians have in recent years meticulously tried to trace to its origin each case or death due to malaria.

The 1943 data which have been assembled (33) show that some of these malaria patients acquired the disease in malarious communities in the South; others were apparently infected while serving in a military or civilian capacity overseas; several were drug addicts who developed the disease through use of a common hypodermic syringe, and a few were apparently infected following blood transfusions. Today persons only infrequently acquire the disease outside of relatively heavy foci of infection in the warmer moist areas of the United States or in malarious territory outside the Continental United States.

THE MALARIA SITUATION TODAY

The several measures which have been undertaken by Federal and state agencies to bring malaria under control have yielded valuable returns, particularly in the heavily endemic areas in the Southern States. Mortality has been reduced by approximately seven-eighths within a decade and it seems likely that morbidity has been decreased to an even greater degree. Whereas it is possible that one out of every hundred persons who contracted *Plasmodium falciparum* infection in hyperendemic areas of South Carolina, Georgia, Florida, Alabama, Mississippi, Louisiana, Arkansas, southeastern Oklahoma and northeastern Texas during the depression years may have succumbed to the disease, it seems equally possible that the present-day ratio of deaths to cases in the same localities ranges between 1:500 and 1:1000. No proof is yet available but the decrease in the number of deaths and the increase in accurate blood-film examination over larger and larger areas provide an increasing amount of qualitative evidence of the interrelationship of these two types of data.

On the basis of this changing malaria picture I have found it helpful to superimpose morbidity data on mortality data for the year 1943 and to plot these on a county map of the United States (fig. 5). This visualizes the *distribution of malaria in 1943* and in no sense indicates the intensity of the disease save in so far as combined mortality and morbidity accentuate the blocks of highly malarious territory. Moreover, there are several apparent inconsistencies in the data. For example, it is quite unlikely that malaria acquired locally in the northern part of the United States (i.e., relatively benign *Plasmodium vivax* infection) was primarily responsible for death. In the second place, it is highly improbable that deaths occurred in the more malarious counties in the South without a corresponding large number of cases of malaria which survived. Again, neither deaths nor cases are recorded for some of the counties which in relatively recent years have been consistently malarious. Nevertheless, the picture as a whole is revealing and, if it is remembered that it represents only a single year's returns and is subject to some fluctuations from year to year, it provides a fairly faithful blueprint of the present-day distributional pattern of malaria in the United States.

THE POTENTIAL DANGERS OF ESTABLISHING EXTRINSIC STRAINS OF MALARIA PARASITES IN THE UNITED STATES

It will be remembered that the indigenous malaria infections of today are those which were introduced by the early settlers from southern Europe and particularly by negro slaves from Africa. These strains of *Plasmodium vivax*, *P. falciparum* and *P. malariae* had little or no difficulty in becoming established in *Anopheles quadrimaculatus* and *A. freeborni* as soon as climatic and local conditions became favorable. The strains of the three parasites have survived the vicissitudes of many decades and for the most part have maintained their virulence, but population groups who have been repeatedly exposed to infection with these organisms have developed relatively high tolerance to the homologous strains. From time to time the infections have become more heavily endemic in the mother foci in the southern United States and soon thereafter have spread rather extensively to adjacent areas. Moreover, *vivax malaria* for many years was also widely distributed in the more temperate northern part of the country where it survived the cold winter months because of its tendency each spring to produce relapse in the infected individuals. This provided the seed for the new crop of *Anopheles* infection. On the other hand the relatively non-relapsing *falciparum malaria* seldom if ever survived the winters in the North and *quartan malaria* was so scant in its endemic foci in the South that it practically never became established in cooler climates. Both vivax and falciparum infections in the inland valleys in California have been repeatedly reinforced by itinerant or seasonal laborers who had acquired malaria in the hyperendemic areas in the South. Thus, there is cumulative evidence to demonstrate that malaria can be readily established anew in *Anophe-*

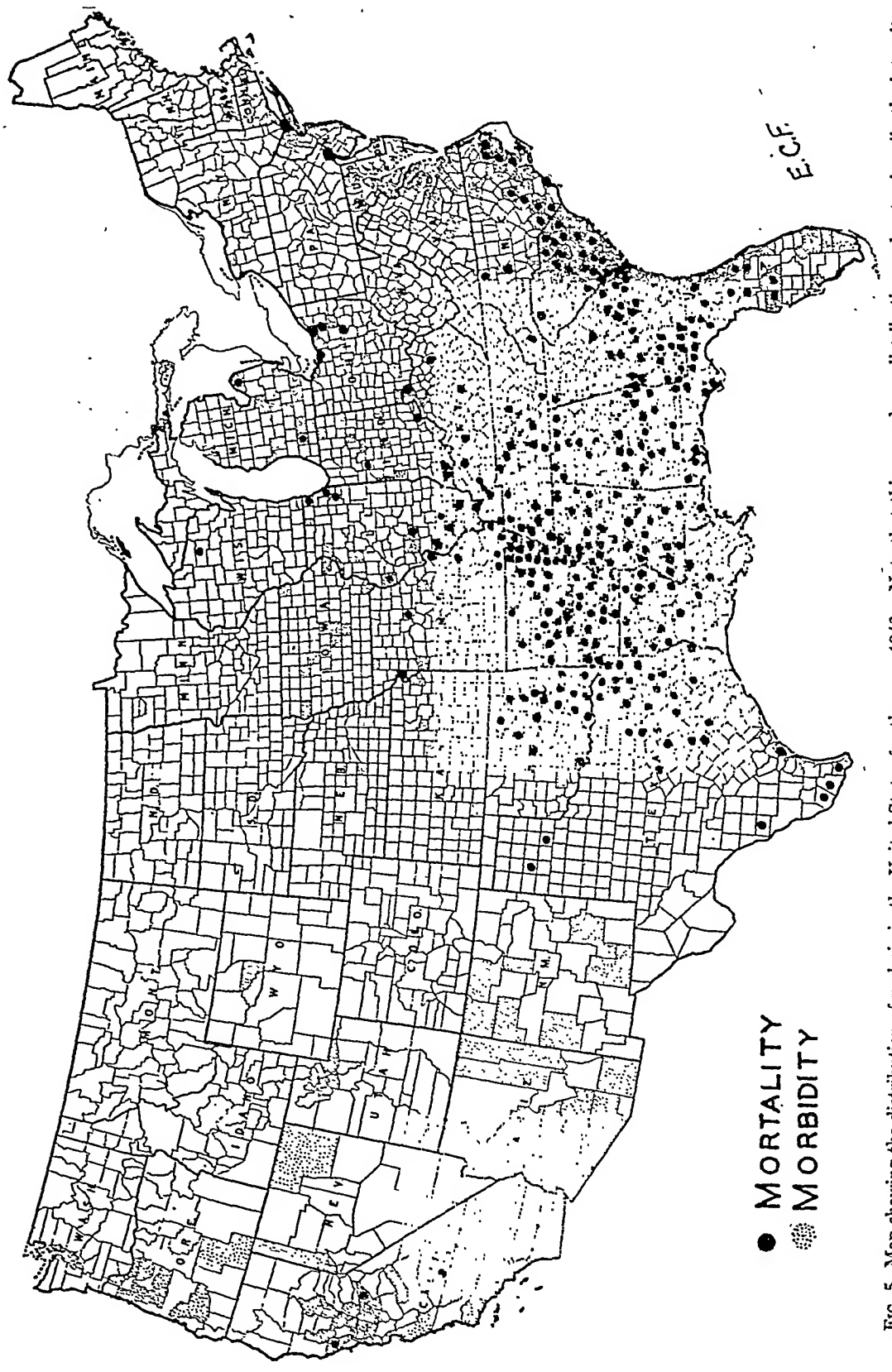

FIG. 5. Map showing the distribution of malaria in the United States for the year 1943. Note that this map shows distribution and not primarily the intensity of infection.

les-breeding areas if favorable conditions of mosquito breeding coincide with sufficient infection in the human population. What are the dangers of establishing new strains of malaria parasites from the Tropics and Mediterranean area with the return of military and civilian personnel who have been exposed in hyperendemic areas?

First of all, it may be stated with considerable assurance (39, 40) that the consistent use of suppressive atabrine therapy, utilizing 1.5 grains (0.1 gm.) of the drug daily per man for six days per week, beginning approximately two weeks before expected exposure to malaria and continuing through the entire period of exposure, has greatly reduced clinical evidence of the infection, irrespective of the species or strain of the malaria parasite. Under disciplined suppressive rationale in *falciparum malaria* it is uncommon for the infection to become either clinically apparent or for the plasmodia to parasitize circulating red blood corpuscles. In consequence *falciparum malaria* rarely appears in returned military personnel from hyperendemic areas. Not only are the immediate incapacitation and the hazards of sequelae largely avoided while the individuals are in the malarious territory, but the danger of introducing these strains into the United States is greatly minimized. On the other hand, *vivax malaria* is characteristically only temporarily kept below the threshhold of clinical expression by suppressive rationale and after cessation of therapy develops to a clinical level and quite regularly produces several to many so-called relapses in spite of repeated use of antimalarial therapy in full clinical accounts. It is this type of patient who constitutes a potential hazard for establishing exotic strains of malaria on his return to the United States; experience is showing that on the average he may constitute a source of potential infection for *Anopheles* mosquitoes for about two years after his return. Thousands of persons with relapsing vivax malaria have already been returned to military hospitals in this country and, in spite of the greatest precautions which are being exercised by medical officers of the Army and Navy, some of these individuals have been and will be sources for mosquito infection in their home communities. Some concrete proof of this statement already exists in the files of state epidemiologists. This danger will continue over a period of time after the last task force has returned from malarious territory.

Civilian personnel and individuals in the U. S. Merchant Marine, who have been in overseas malarious areas and have not been subject to the exacting discipline of suppressive therapy now carried out by our military establishments, constitute a less common source but a more likely one for the introduction of malaria into this country. Moreover, several of these infected individuals have already been seen, particularly in ports of the Atlantic Seaboard, where charity clinics, board of health laboratories or private physicians have been consulted. This group has been found to harbor all three species of malaria parasites, so that these returning civilian and Merchant Marine personnel, who have been under no military discipline or regulations, constitute a relatively important potential source of new malaria.

In connection with the importation of malaria there remains the important question whether these strains of plasmodia are acceptible to our malaria-transmitting mosquitoes. Without waiting for long-time practical demonstrations it has already been ascertained that *Anopheles quadrimaculatus* and *A. freeborni* will develop strains of vivax plasmodia from Mediterranean and Southwest Pacific foci as readily as these mosquitoes do the strains well-established in this country (41). Thus, there is no natural obstacle at least to the establishment of exotic strains of vivax malaria in the United States. This demonstration suggests the need for even greater precautions to keep gametocyte carriers of exotic strains of vivax malaria from native anopheline mosquitoes.

SUMMARY

There is essentially no evidence that malaria existed in the United States before the advent of European explorers and settlers. It became a serious clinical and public health problem with the importation of African slaves for rice cultivation in the Carolinas and sugar cane development in the Gulf Coast plantations. Its establishement was made possible by bringing together readily available sources of infection for mosquitoes and by susceptible *Anopheles* mosquitoes which increased in numbers with the breaking of virgin sod and felling of forests. From the primary foci it spread along the Atlantic Coast from Florida to New England, was carried along several routes over the Appalachians and became well established throughout practically the entire Mississippi drainage. It was likewise carried by explorers and immigrants to the Pacific Coast, where it developed substantial roots.

By 1850–1860 most of the settled part of the

country was highly malarious, although the hotbeds of the infection were in the South. Federal forces in the South suffered heavy casualties during the military operations of the War Between the States, while the Days of Reconstruction visited upon the South a prolonged period of intense malariousness. Although there was some improvement during the last two decades of the nineteenth century, even the discovery of the etiological agents of malaria and the elucidation of their life cycles, with the essential role played by *Anopheles* mosquitoes, did not greatly stimulate accurate diagnosis or the undertaking of control measures. Yet little by little as prosperity developed in the United States, as land came under more intensive cultivation and the price of quinine was greatly reduced, the heavily endemic territory began to shrink into the area from the coast of Virginia to Central Florida and westward to eastern Oklahoma and Texas.

Between 1915 and 1933 there was little change in the malaria picture except for the five-to-seven year cycles of ebb and flow. The serious economic depression beginning in 1931 greatly increased the amount and distribution of the disease, but this stimulated extensive coordinated control measures which have been responsible, at least to a considerable degree, for the marked reduction in malaria mortality and morbidity. Yet today there is extensive malaria in the South, although much less intensive than a decade ago.

In contrast to the situation which existed during the Civil War and the Spanish-American War, during World War I Army encampments in the South provided careful diagnosis and treatment for their malaria patients and established the first reliable actuarial basis between deaths and cases in the malarious Southern States. With much more experience, during World War II the malaria case rate in soldiers who had not been outside of the Continental United States has constituted a small fraction of that in the World War I in the same endemic foci. This has been due to military efforts within the posts and those of public health agencies immediately around the camps. Moreover, this joint control has also had its favorable effects on the nearby civilian communities.

Because of suppressive chemotherapy in hyperendemic malarious areas of military operations outside the Continental United States, materially aided by malaria survey and control operations, malaria is no longer a serious clinical problem within controlled military zones. Moreover, falciparum malaria is usually liquidated by adequate suppressive treatment, but vivax malaria is characteristically subject to repeated relapses up to two years. Thus, vivax cases among military personnel returning to the United States constitute a potential hazard for the establishment of exotic strains throughout the country wherever susceptible *Anopheles* mosquitoes are allowed to breed. In addition, civilians and merchant seamen, who have not been under strict military discipline and have been exposed to malaria overseas, constitute a grave potential risk since they frequently harbor falciparum, vivax and even at times quartan malaria parasites.

CONCLUSIONS

At the lowest level of malaria in our history since the disease became firmly established nearly two hundred years ago there is the opportunity to reduce the infection below the level of natural propagation, provided the following measures are carried out:

1. Reduction of *Anopheles quadrimaculatus* and *A. freeborni* breeding in hyperendemic foci to a point where the chances of man-mosquito-man development of the life-cycle are very infrequent.
2. Continued, more extensive consciousness of malaria in patients appearing for consultation and treatment, not only in highly endemic territory but in areas of milder endemicity and in non-malarious regions.
3. Increased accurate blood-film diagnosis.
4. Treatment of all malaria patients with adequate amounts of antimalarial drugs until cure is effected.
5. Inquiry by all physicians consulted by veterans, merchant seamen or civilians, who have been overseas, regarding a history of malaria or exposure to malaria, and notification to local health authorities of potential malaria carriers.
6. A minimum food ration for the poorer elements of our population, which is quantitatively and qualitatively adequate to produce resistance to disease. (Frequently actual cure can not be effected until bodily resistance has been built up to destroy the malaria parasites in the foci outside red blood cells.)
7. Serious consideration of the advisability of instituting suppressive antimalarial therapy to populations in hyperendemic foci which

remain resistant to reduction by other practical methods.

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LABORATORY STUDIES OF THE SAIMIRI-HAEMAGOGUS CYCLE OF JUNGLE YELLOW FEVER[1]

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I. INTRODUCTION

In a recent article, Drs. Bugher, Boshell-Manrique, Roca-Garcia and Osorno-Mesa (1944) published the results of their extensive field investigations of yellow fever in eastern Colombia. On the basis of these studies they formulated a theory of the epidemiology of the disease which is, essentially, that virus is maintained by constant mammal to mammal passage by means of mosquito vectors, chiefly a single mosquito species, *Haemagogus capricornii*. This theory would have a sounder basis if the transmission mechanisms postulated from the field evidence could be reproduced and studied under conditions of laboratory control, and our efforts during the past several years have been largely directed toward this goal. It seems surprising that the laboratory reproduction of possible transmission mechanisms in yellow fever in Colombia should be difficult, since transmission of the virus by *Aedes aegypti* and other mosquitoes between rhesus and other monkeys has long been commonplace. We found, however, unexpected difficulties when we tried to maintain such transmissions with entirely local materials—with local mammals, local strains of virus and local mosquitoes. The chief obstacle seems to have been difficulty in handling the vector mosquito (*Haemagogus capricornii*) in the laboratory, but it was also necessary to make studies of the behavior of various strains of virus and of various mammal species under the experimental conditions.

We have long thought that if we could get one local transmission mechanism under laboratory control, the study of other possible mechanisms would be greatly facilitated and a standard would be established for the evaluation of various mammals as hosts and various mosquito species as vectors. The object of the present article is to describe such a "standard mechanism" which, at the time of writing, has been carried through 5 laboratory cycles, with saimiri monkeys as hosts and *Haemagogus capricornii* as vectors, so that there is no question as to the reproducibility of the particular virus-mosquito-mammal relationship. The earlier transmission experiments have not been reviewed because it is difficult to determine whether particular results were properties of the materials used (mammal, mosquito, or virus strain) or of the technical procedures. Experiments have been started using mammals other than saimiris and mosquitoes other than *Haemagogus capricornii*, but this work is still in a preliminary stage. Studies of virus behavior in saimiri monkeys have already been published (Bates, 1944a), and attention in the present review is consequently centered on the mosquito half of this particular transmission cycle.

The experiments on virus behavior in the mosquito would have been much clearer and more satisfactory if parallel experiments could have been made with *Aedes aegypti*. This species does not, however, occur in the region of the laboratory, and we have not cared to run the risk of possible escapes by introducing a laboratory colony.

The first attempts to obtain transmission of yellow fever virus with *Haemagogus capricornii* (*janthinomys*) were made by Kumm and Frobisher (1932), who were unable, however, to keep the mosquitoes alive long enough to complete their experiments. Antunes and Whitman (1937) have reported a series of experiments with species of the genus *Haemagogus*. They found that the mosquitoes retained virus for at least two weeks, and they secured transmission in one instance. They also experienced difficulties in maintaining the mosquitoes alive in the laboratory. Virus was first isolated from wild specimens of the genus by Shannon, Whitman and Franca (1938), who obtained transmission to a rhesus monkey by bite by a captured *Haemagogus capricornii*. Virus has subsequently been isolated repeatedly from this species in Colombia by Bugher and his co-workers (1944).

The genus *Haemagogus* presents difficulties from the taxonomic point of view, and the various

[1] The studies and observations on which this paper is based were conducted with the support and under the auspices of the Section of Special Studies maintained by the Colombian Government and the International Health Division of The Rockefeller Foundation.

specific populations are distinguishable only by rather obscure morphological characters. The problem of identification is simplified in the Villavicencio area by the apparent homogeneity of the population; many thousands of specimens have been examined by Komp (1936), Antunes (1937), and in the course of the present work by Drs. Kumm, Boshell, Osorno and the writers, but only a single specimen with anomalous morphological structure has been discovered. The taxonomy of the various populations that have so far been discovered in Colombia has been reviewed by Kumm, Osorno and Boshell (to be published), who assign the Villavicencio population to the species *Haemagogus capricornii*. The term "haemagogus" in the present paper refers always to this particular population.

The writers are indebted to their colleagues for the stimulus of a keen interest and for many helpful suggestions: particularly to Dr. C. R. Anderson for suggestions regarding virus behavior, and to Drs. H. W. Kumm, J. Boshell-Manrique and E. Osorno-Mesa for suggestions regarding mosquito techniques. Primary credit for realizing the possibilities of the method of maintaining haemagogus adults isolated in individual tubes should go to Dr. Boshell; this technique is the key to successful experimentation with these mosquitoes.

II. MATERIALS AND METHODS

Mosquitoes

In most of the experiments wild-caught mosquitoes were used. This material was collected in the course of field studies of haemagogus distribution and behavior and brought to the laboratory for identification. These field routines have been described in previous papers (Bates, 1944c and 1945). There has been no indication of the presence of virus in these study areas since 1940. In 1942 large numbers of mosquitoes from the areas were inoculated in mice without in any case recovering virus, and no evidence has appeared in the present experiments that would indicate contamination with an outside virus. The mosquitoes were brought to the laboratory in individual test tubes, identified, and stored in the separate tubes at 20° or 25°C. until wanted for experimental purposes; they were always used within 48 hours of capture, as it is impossible to maintain them longer without food.

The mosquitoes, still separate in test tubes, were allowed to feed on the source animal one at a time, in the case of saimiri monkeys most frequently on the palms of the hands and feet, but sometimes on the shaved abdomen. We found attempts to feed groups of hemagogus mosquitoes in cages relatively unsatisfactory. The engorged mosquitoes were kept in the tubes until a particular infection series had been completed. At the end of the feeding the mosquitoes that had failed to engorge were destroyed, and a small number of engorged specimens (usually 3 to 5), taken at random, were inoculated intracerebrally in adult mice as a check on the ingestion of virus. The source animal was also bled immediately after the feeding, and decimal dilutions of serum were inoculated in adult mice as a check on virus circulation.

The infected mosquitoes were maintained in individual flat-bottomed vials about 25 × 50 mm. in size, a modification of the method first devised by Barber and Shannon for isolating *Anopheles maculipennis* and described by Hackett and Missiroli (1935). We have experimented with many modifications of this technique and have found that haemagogus survival may be influenced by apparently trivial details. The method used in the experiments reported on here is shown in figure 1. The vials have a layer of moist absorbent cotton in the bottom, covered by a disc of filter paper (to prevent the mosquitoes' getting entangled); they are plugged with cups of aluminum wire screening, which contain a bit of absorbent cotton which is moistened on alternate days with a weak sugar solution. The vials are kept in racks that hold 22 specimens, as shown in the figure; each vial is labelled with a bit of adhesive tape bearing the number of the experiment and the number of the individual mosquito. In this way, the history of a single mosquito can be followed with relative ease. At first we transferred the mosquitoes to clean vials fairly frequently, but this seems unnecessary if care is taken to maintain the moisture in the bottom layer of cotton. This is very important; mortality increases at once if the cotton is either too wet or too dry, and the proper balance is a question of nice judgement and constant watchfulness.

All mosquito manipulations were carried out in a small screened corner of the infected mosquito room (which itself was well isolated from the rest of the laboratory). In this way, mosquitoes that escaped in manipulating the tubes could be quickly recaptured. Since records were kept

on individual mosquitoes, any losses were at once apparent. When a mosquito was to be used for transmission, the vial was unplugged and inverted over the skin of the animal which was to serve as host, and the mosquito was allowed to feed.

Temperature

The term "constant temperature" as used in this paper is somewhat relative, because of the uncertainty of the local electric plant and the crudeness of some of our homemade apparatus. Thermographs were maintained with all lots of infected mosquitoes, so that we at least have a record of the irregularities. The following temperature conditions were used in connection with the present study:

20°C.: This was obtained in a cabinet with freezing and heating units balancing each other; temperature control was normally very exact except when current was not available (usually for a few hours about once a week), at which times the temperature might rise to 22° or 23°.

30°C.: The temperature in this cabinet generally varied between 30° and 31°, rarely rising to 32° or (when the current failed) falling to 28°.

Room temperature: Room temperatures, by northern standards, are very regular in our laboratory. During the time when the experiments reported here were made, the mean was 25.4°C., the absolute minimum 23.5° and the absolute maximum 28°. These were exceptional swings of the thermograph pen; the daily fluctuation was usually between 24° and 27°, and most of the time the mosquitoes would have been living at a temperature of 25° or 26°.

Saimiri monkeys

Wild-caught, local animals were employed in all experiments. The characteristics of these animals and methods of handling them have already been described (Bates, 1944a). Only animals whose sera were previously found negative in the yellow fever protection test were used. They were bled again immediately before use, and the sera were held in reserve for testing in case of unusual behavior. The animals remained isolated in the infected animal room from the time they were used in an experiment until death or until they were bled for a postinoculation protection test, usually 30 days after exposure to virus. They were tested daily from the 3rd to 7th day for circulating virus by inoculating serial decimal dilutions of serum in adult white mice. Rectal temperatures were taken morning and evening for 10 days after exposure to mosquitoes. At death autopsy was performed. Liver tissue was always preserved for histological examination.

FIG. 1. Method of keeping individual infected mosquitoes isolated in shell vials

Mouse techniques

The testing of material for the presence of yellow fever virus by the intracerebral inoculation of

white mice has become a routine procedure since the publication of Theiler's paper (1930). The methods of inoculating (0.03 cc. of suspensions) and handling mice and of reading mouse results used in the present work were essentially similar to those described by Bugher (1940) for protection test routines. Mice were invariably observed for 19 days after inoculation, and often for much longer periods. Mosquitoes for mouse inoculation were ground separately, without abrasive, in 0.5 cc. of 10% normal human serum in distilled water. The suspension was inoculated without previous centrifugation.

We made extensive use of baby mice in these studies because of their greater susceptibility to infection with the virus of yellow fever. Baby mice used for intracerebral inoculation were generally 5 days old; mice used for mosquito transmission experiments were always 3 days old. The methods of handling such mice have been described by Bugher (1941). Where parallel inoculations were made of the same material in baby and adult mice, the same syringe was used, the group of baby mice being inoculated first. Adult mice were generally 42 to 60 days old, but in a few instances when such mice were not available, younger animals were used.

All protection tests of monkey sera reported in this paper were made in the Villavicencio Field Laboratory, using the intracerebral technique described by Bugher (1940).

III. TRANSMISSION OF VIRUS AND MAINTENANCE OF CYCLES

Transmission of virus by haemagogus that had been infected on saimiri monkeys was obtained several times in 1943 (Bates, 1944a); but positive results were irregular, and attempts to maintain cycles of transmission failed. These experiments were made with strains of virus that had been subject to considerable laboratory manipulation (the African Asibi, and the local Martinez and Novoa strains), and we felt that it would be better to use a strain of virus recently isolated. The strain which we used was obtained from a patient (Carlos Perez), who died of yellow fever contracted in the Restrepo area north of Villavicencio. Serum of this patient was inoculated intracerebrally in adult mice, and when some of these showed signs of paralysis, brain suspension was passed directly to a saimiri monkey. Serum of this monkey was desiccated and used to infect a new monkey 3 months later when we were ready to start experiments. The virus was maintained by mosquito-monkey passages through 5 complete cycles, as shown in the accompanying diagram (fig. 2). This diagram includes only monkeys and mosquito lots that were proved to have virus.

Virus transmission by haemagogus

The histories of all saimiris that were fed on by infected haemagogus in the course of these experiments are summarized in Table I. The results of intracerebral inoculation of groups of baby and adult mice with tissue suspensions of mosquitoes killed immediately after feeding individually on baby mice, are summarized in Table II. In the case of saimiris, mosquitoes from various temperature experiments are included in the table; in the case of baby mice, only mosquitoes that had been kept at a constant temperature of 30° are included.

It will be noted that at room temperature we clearly failed to get transmission at 10, 14, 15 and 20 days; we apparently failed to get transmission once at 24 days (saimiri 113); we got delayed infection (saimiri 112) at 22 days, and acute infections at 24 and 32 days. It seems from this that the "extrinsic incubation period" under the circumstances of these experiments (mosquitoes infected on saimiris circulating "large" amounts of this virus strain, and kept at a fluctuating temperature of 24°–27°C.) is between 22 and 24 days.

With experimental conditions similar, except that the mosquitoes were kept at 30°, we secured an atypical infection in a saimiri (no. 137) in one case at 10 days, and acute infections after more than 14 days. The earliest transmission to a baby mouse was at 13 days, and transmission was obtained regularly from mosquitoes that showed virus at 17 days or more. We judge from this that the extrinsic incubation period at this temperature is 13–15 days.

Davis (1932) summarized experiments with the effect of temperature on extrinsic incubation period in *Aedes aegypti* infected with Asibi virus. The incubation periods were in all cases much shorter than those reported here. He secured transmission at 6 and 8 days at 30°–31°, at 11 days at room temperature (22°–25°) and at 18 days at 21°; transmission was not secured with mosquitoes kept at 17°–19° (periods up to 30 days), but virus was retained by these mosquitoes and transmission

was obtained by keeping them for 6 days longer at room temperature. Whitman and Antunes (1938) have shown that extrinsic incubation period is in part, at least, a function of the strain of virus: they found that the incubation period in *Aedes aegypti* infected with jungle strains is about 4 days longer than in those infected with the Asibi strain (room temperature, mean 24.5°C.). The single transmission reported by Antunes and Whitman (1937) with *Haemagogus capricornii* (*janthinomys*) was obtained after 14 days, but temperature conditions were not specified.

Characteristics of infection in saimiri monkeys

General observations on the behavior of local virus strains in saimiri monkeys have been given in a previous paper (Bates, 1944a). Inspection of Table I shows that 10 of the 18 monkeys fed on by mosquitoes became infected; of these 10, 8 died between the 3rd and 6th day after being bitten. The 2 that failed to succumb (137 and 154) showed mild infections, atypical in several respects. Saimiris are small and rather delicate animals, and the treatment that they receive in

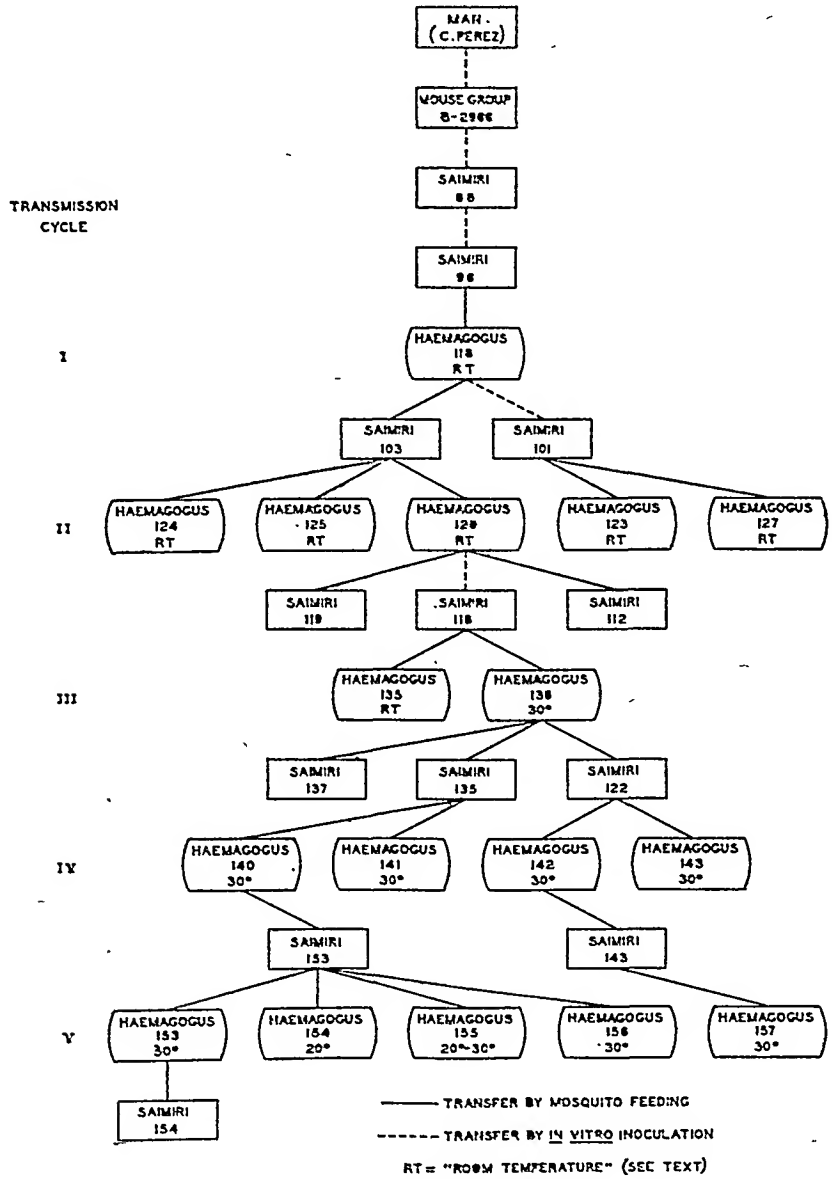

FIG. 2. Diagram of passages of Perez virus

the course of the experiments is rather severe, particularly the daily bleedings in which 2 cc. of blood is usually taken from the femoral vein. Yet these deaths must presumably be ascribed to yellow fever infection, since there have been no deaths in the uninfected animals receiving similar treatment. Signs of haemorrhage, particularly in the stomach, were frequently found at autopsy. There is some evidence that this virulence is an adaptive character of this strain of virus, since the first 2 monkeys in the passages from man (nos. 88 and 96 of fig. 2) did not die, even though they showed high titers of circulating virus. It is notable that another strain with which we are currently working, and which was inoculated into a saimiri monkey directly from a human patient with a non-fatal case of yellow fever, has given rise to fatal infections in saimiris from the beginning.

"Febrile reaction" in Table I means that the animal showed a sustained temperature of over 39°, including interruption of the daily alternation of morning low and evening high temperatures.

TABLE I

Histories of saimiri monkeys bitten by haemagogus from lots known to have been infected

SAIMIRI NO.	HISTORY OF MOSQUITOES				HISTORY OF SAIMIRI						
	Lot no.	No. biting	Days inf.	Temp.	Circulation of virus (days after being bitten)					Febrile reaction	Postinoculation protection test
					3	4	5	6	7		
97	118	7	10	R	—	—	—	—		No	Neg.
100	118	13	15	R	—	—	—	—	—	No	Neg.
103	118	8	24	R	+	+	+	†		Yes	
108	126	16	10	R	—	—	—	—	—	Yes	Neg.
109	126	19	14	R	—	—	—	—		No	Neg.
94	126	6	20	R	—	—	—	—	—	No	Neg.
112	126	4	22	R	—	—	—	+†		Yes	
113	124	3	24	R	—	—	—	—		No	Neg.
119	126	3	24	R	—†					Yes	
132	127	2	32	R	+	+	+†			No	
137	136	4	10	30°	—	+	—	—		No	Neg.*
135	136	10	14	30°	—	+	+	+†		No	
122	136	5	18	30°	+	+	+†			No	
128	140	5	13	30°	—	—	—	—	—	Yes	Neg.
153	140	5	21	30°	+	+	+†			Yes	
143	142	10	25	30°	+	+†				Yes	
154	153	4	19	30°	—	—	—	—	+	No	Neg.‡
166	157	2	20	30°	—	—	—	—	—	No	Neg.

"R" signifies "room temperature" (24°–27°, mean 25.4°C.); "†" indicates death of monkey.

* The history of saimiri 137 is very curious. It showed traces of virus in the 1:2 and 1:10 dilutions of serum inoculated intracerebrally into mice on the 4th day; brain suspensions of these mice were passed to new mice, and a specificity test was positive for yellow fever; virus was not again recovered. The animal was bled twice for postinoculation protection tests, and each serum was tested twice in different runs; the results showed either no protection or slight protection. The monkey was reinoculated 2½ months after this first infection with 7,000 m.l.d. (for mice) of a mouse-adapted subline of Perez virus (6 mouse-brain passages); it showed no febrile reaction and survived the reinoculation with no apparent ill effect; but *virus was found in circulation on the 3rd and 4th days after inoculation.* A normal saimiri inoculated in parallel with no. 137, with the same dose from the same syringe, showed an acute infection with fever, dying on the 5th day after inoculation. We should like to believe that a mistake was made somewhere in this history, but while this possibility cannot be eliminated, it seems remote. It looks as though the animal had received a subinfectious dose from the mosquitoes, and thus had acquired resistance, but not immunity, to subsequent infection.

‡ Saimiri 154 showed a trace of virus on the 7th day; it died of some unknown cause on the 14th day. Serum taken post mortem showed no protective power as measured by the intracerebral protection test. Both of these monkeys seem to be examples of atypical infections following bites by mosquitoes transmitting traces of virus.

It will be noted that this proved to be an unreliable guide to infection in the animals; in some cases the animal, instead of fever, showed a steady drop

TABLE II

Transmission experiments with Haemagogus capricornii in which mosquitoes that had been kept at a constant temperature of 30°C. were killed immediately after feeding on baby mice and suspensions in 0.5 cc. serum-water were inoculated intracerebrally in parallel groups of baby and adult mice

LOT AND MOSQUITO NO.	DAYS AFTER INFECTIOUS MEAL	MORTALITY RATIOS OF MOUSE GROUPS INOCULATED I.C.		TRANSMISSION BY BITE TO 3-DAY-OLD MOUSE
		Babies	Adults	
153-4	10	5/5	3/5	–
11	10	4/4	6/6	–
14	10	5/5	4/6	–
25	10	5/5	2/6	–
34	10	3/5	0/6	–
5	13	5/5	0/6	–
12	13	0/5	0/5	–
13	13	5/5	4/6	+
27	13	0/5	0/6	–
46	13	5/5	2/6	–
49	16	1/4?	0/3	–
28	16	0/4	0/5	–
36	16	0/3	0/6	–
143-21	17	0/2	0/6	–
23	17	4/4	2/6	+
24	17	0/4	0/6	–
27	17	1/2	0/6	–
29	17	4/4	1/6	+
54	17	4/4	1/6	+
45	17	0/2	0/6	–
60	17	0/4	0/6	–
142-20	18*	5/5	2/5	+
15	18	2/4	1/6	–
10	18	5/5	5/5	+
25	18	0/4	0/5	–
143-9	20	4/4	2/4	+
20	21	4/4	2/6	+
33	21	4/4	5/6	+
22	21	4/4	1/5	–
39	21	4/4	4/6	+
142-34	31	5/5	6/6	+
9	31	4/4	3/6	+

* These four mosquitoes were kept for 18 days at 30°, then transferred to 20° for 12 days more; as we have no evidence of virus multiplication at 20°, they are listed here as "18 days after infectious meal."

in temperature starting on the 2nd or 3rd day and continuing until death. Death was usually preceded by a period of coma that might last for 12 to 24 hours.

Maintenance of cycles

The method of serial transmission from mammal to mammal through mosquitoes is valuable in that it insures that the properties of the virus strain are not being changed by "unnatural" manipulation, and as a concrete demonstration of the possibilities of a particular mechanism in nature. But with animals like haemagogus mosquitoes and saimiri monkeys it is not a very practical method of virus maintenance, in so far as it requires a considerable expenditure of effort and materials. The chief difficulty, perhaps, is in judging when to feed new mosquitoes on a possibly infected saimiri. We fed fresh lots of mosquitoes on all of the saimiris used in the experiments, and since many of these monkeys proved not to have been infected (Table I), this material was wasted. The most practical system seemed to be to feed the mosquitoes on the 4th and 5th days after possible infection, though in this way we sometimes missed feeding the mosquitoes on the day of maximum virus circulation. We reached the conclusion that for the study of special problems of virus behavior in mosquitoes it would be better to infect the mosquitoes on monkeys inoculated with known amounts of virus, as the course of infection in the monkey can then be predicted with a high degree of probability. For tests of mosquito transmission the method of using baby mice (Bugher, 1941) has great advantages in so far as large numbers of mosquitoes can thus readily be tested as individuals.

IV. BEHAVIOR OF THE VIRUS IN WHITE MICE

White mice have been inoculated at every stage in these experiments to detect the presence of virus, and while the problem of mouse susceptibility to the virus of yellow fever is foreign to the main object of this article, some comment on the behavior of the Perez strain on mouse inoculation is necessary. The virus was passaged through mouse brain only once in the history of the strain (see diagram, fig. 2), and this single passage does not appear to have increased the neurotropism of the strain. The infection of adult mice by intracerebral inoculation has been irregular, and to a certain degree unreliable, throughout the experiments. The unreliability of the response of adult mice to some jungle (pantropic) strains of virus is familiar to everyone who has worked with such strains, though little has been published on the subject. Fox (1943) in a brief review of the

behavior of jungle strains as compared with the similar behavior observed in some of the vaccine strains (17D sublines) states that "as the result of experience with more than 100 jungle strains that have been isolated up to the present time, workers in Brazil have long recognized that the susceptibility of mice to unmodified virus of jungle origin may not be uniform. Freshly obtained serum from yellow fever patients has often been observed, when inoculated into mice, to give rise to a mild disease from which the animals recover completely if given the opportunity."

The difficulty with the interpretation of mouse results led us to make many experimental checks that would otherwise have been unnecessary. Infection in our strain of mice became regular after 2 or 3 brain passages (particularly if in baby mice) and by this means specificity tests (by parallel titrations with known normal and immune sera) were made with the infectious agent from every saimiri classified as "infected" and with material from a large proportion of the mosquitoes, in every case with positive results. The incubation period of the virus in adult mice varied from 9 to 14 days or more, depending largely on the amount of virus inoculated; irregular mortality was in part, at least, a quantitative phenomenon, being most pronounced with small amounts of virus, as in mosquito suspensions. As a result of this, titration by the intracerebral inoculation of adult mice has been of doubtful significance with this strain, and in consequence no specific virus titers are given in this paper for either monkeys or mosquitoes. The unreliability of such titrations in some strains is well shown by the results tabulated by Fox (1943, p. 518) for estimates based on "deaths only" (the usual procedure) and on "deaths plus non-fatal infections."

We tested many surviving mice with challenge doses of "French neurotropic virus" (Fox, 1943), with variable results. Mice which had survived inoculation with virus-containing materials certainly in many cases were resistant on reinoculation, but the correspondence seemed irregular and incomplete. More regular results were obtained by making protection tests (intracerebral) with the sera of surviving mice; by this means it was possible to demonstrate immunity in all cases where we had reason to suspect that the mice had survived a virus inoculation.

The relative resistance to infection by intracerebral inoculation was characteristic of adult mice (42 or more days old), and we found that 5-day-old mice were much more uniformly susceptible. In later experiments (the 4th and 5th cycles) all attempts to recover virus from mosquito material were made with parallel inoculations in adult and baby mice. The different response of the two age classes can be seen by an inspection of the results included in Tables II and III and discussed later in this paper.

TABLE III

Distribution of "mortality ratios" of mouse groups inoculated with suspensions of individual mosquitoes 10 or more days after infectious meal; mosquitoes kept at a constant temperature of 30°C.

A. Groups of 5-day-old mice

Mouse mortality	0/4 0/5	1/4 1/5	2/4 2/5 3/5	3/4 4/5	4/4 5/5
No. instances	10	1	2	0	27

B. Groups of adult mice

Mouse mortality	0/5 0/6	1/5 1/6	2/5 2/6	3/6	3/5 4/6	4/5 5/6	5/5 6/6
No. instances	17	5	5	4	5	2	6

V. BEHAVIOR OF THE VIRUS IN HAEMAGOGUS CAPRICORNII

The evidence indicates, in the case of animal viruses that have been carefully studied, that transmission by a vector mosquito represents an infection of the mosquito by the virus. There is no evidence of pathological lesions in the mosquito host, or of deleterious effect from such an infection; but the prime criterion of virus infection is multiplication. Evidence for such multiplication in mosquito tissue has been given by Merrill and Tenbroeck (1935) and Trager (1938) for equine encephalomyelitis and by Whitman (1937) for yellow fever. It might be noted that the evidence for true infection in the case of insect vectors of plant viruses is not so clear (reviews by Storey, 1939, and Leach, 1940); the insects, in some cases

at least, may be merely agents of transference for the virus from host to host. In the case of vector mosquitoes and the virus of yellow fever, however, it would seem that we may legitimately speak of the factors which govern the infection of the mosquito; and the whole virus mosquito relationship seems clearer if it is thought of in such terms. The principal factors governing such infection appear to belong to four groups: the characteristics of the virus, the characteristics of the mosquito, the virus dosage ingested and the environment of the mosquito.

In the series of experiments under review, we attempted to keep the first two factors constant (virus strain and mosquito species). We must, of course, keep in mind the possibility of change in virus characteristics through "adaptation" in the course of the experiments, and the possibility of genetic variation in the mosquitoes, though there is no evidence that either of these factors has influenced the experimental results to an extent that would vitiate comparisons between experiments within the series. The present analysis, then, is an attempt to describe the behavior of a particular local strain of virus in the local haemagogus population, under circumstances in which there are two variable factors—the amount of virus ingested and the environment of the mosquito.

Percentage of mosquitoes infected

The impression that we have had from all experiments with haemagogus (those of previous years as well as those of the present series) is that under a given set of circumstances only a certain percentage of the mosquitoes becomes infected. This may well be generally true of mosquitoes infected with yellow fever virus, but in published experiments it is generally masked by the usual method of grinding and injecting pools of mosquitoes. The point that not all mosquitoes become infected under a given set of circumstances is sufficiently important to warrant giving two examples of what is meant before proceeding with the discussion.

Fifteen mosquitoes of the 1st transmission cycle (lot 118) were still alive on the 24th day after infection; these were killed and inoculated separately in adult mice; virus was recovered from 5 of the 15 (33%). The mouse mortalities were, respectively, 3/6, 3/6, 3/6, 4/6 and 5/6 for mosquito suspensions in 0.5 cc. of serum-water. The gap between 0/6 and 3/6 mortalities with lot 118 is rather unusual in inoculations of adult mice, but it is probably significant and due to the fact that these mosquitoes were inoculated a long time (24 days) after ingesting a large virus dose.

A better indication of virus recovery may be obtained from an analysis of the results from the inoculation of baby mice. If it is true that the mosquitoes fall into two discrete groups, those that become infected with virus and those that fail to become infected, the distribution of the mortality of mice inoculated with mosquito material (after a suitable extrinsic incubation period) should be bimodal, with peaks at 0% (0/6 mortality) and 100% (6/6 mortality). To test this, we selected a homogeneous group of inoculations: all mosquitoes killed 10 days or more after infection that had been kept at a constant temperature of 30°C. Suspensions of such mosquitoes were routinely inoculated into groups of 6 adult mice and parallel families of 4 or 5 baby mice. Results were rejected where more than 1 mouse died from trauma or intercurrent infection. This restriction gave us a group of 40 mosquito inoculations in baby mice, and of 46 inoculations in adult mice. The distribution of these results is shown in Table III. The results of the adult inoculations show a random distribution, but the baby mouse results fall into two classes, no mortality or 100% mortality, with only 3 intermediate results in 40 instances. The results of some of the individual parallel inoculations used in preparing Table III are included in Table II above.

The percentage of mosquitoes infected (i.e., showing virus on mouse inoculation) varied from experiment to experiment; under the conditions of this study (virus strain and mosquito population uniform) this variation might be influenced by three factors: time (extrinsic incubation period), temperature, and virus dosage ingested.

Effect of time

In the 2nd cycle of transmissions there were 5 lots of mosquitoes with more or less similar histories (lots 123–127 of fig. 2); we did not wish to endanger the maintenance of the virus strain by killing significant numbers of these mosquitoes, so we inoculated all mosquitoes that were found dead in the tubes from the 10th day after infection on. The mosquitoes were examined twice daily (8 A.M. and 4 P.M.) and the dead mosquitoes were removed, ground in 0.5 cc. serum-water each, and inoculated in adult mice. This method has two serious drawbacks: first, the length of time the mosquito has been dead is unknown and virus

may not have survived in the tissues; second, the response of adult mice to small dosages of this pantropic virus strain is irregular. Nevertheless indications obtained from this experiment have been substantiated by subsequent work, and as a matter of fact, results from the inoculation of dead mosquitoes were found to be surprisingly similar to results with mosquitoes freshly killed.

If the separate cases of virus recovery in this series of inoculations are charted according to days after infection, the positives seem to be completely scattered. This has been summarized in Table IV. The 50% recovery in the 25–30 day period represents a very small sample (6 of 12 mosquitoes) and should be judged in the light of the 12% recovery from the previous period (20 to 24 days). Certainly there is no pronounced trend of recovery of virus from increasing numbers of mosquitoes with the passage of time.

TABLE IV

Recovery of virus from inoculation of suspensions of dead mosquitoes into adult mice (lots 123–127, kept at room temperature)

DAYS AFTER INFECTION	NO. OF MOSQUITOES INOCULATED	NO. SHOWING VIRUS	PER CENT POSITIVE
10–14	33	7	21
15–19	31	9	29
20–24	26	3	12
25–30	12	6	50
Totals.....	102	26	25

This same scatter effect has been observed in all subsequent experiments. Studies have not been made during the first 10 days after infection, but considerable variation would no doubt be found in this period as the ingested virus either died or became established in the mosquito tissues. This has been demonstrated by Whitman (1937), who failed to recover any virus from *Aedes aegypti* infected with the Asibi strain 3 and 4 days after infection. The point that recovery of virus, after the 10 day lapse, is not a function of time becomes important in evaluating the effect of temperature, discussed below. It may be well to cite one more example from lot 153 (fifth cycle, kept at 30°), in which groups of mosquitoes were killed and inoculated in both adult and baby mice at 3-day intervals. At 10 days 5 of 5 mosquitoes showed virus; at 13 days, 3 of 5; at 16 days 1 of 3 (individual results at these periods are listed in Table II); at 19 days, 3 of 7. In this case, the highest percentage of recovery was from the mosquitoes first inoculated!

Effect of temperature

In the third transmission cycle, the mosquitoes that fed on saimiri 118 were divided, at random, into two equal lots of 33 mosquitoes each; one of these lots (no. 135) was kept at room temperature (24°–27°C., discussion under "methods" above); the other (no. 136) at a constant temperature of 30°. Dead mosquitoes were inoculated in adult mice from the 10th day on, as before. From lot 135, kept at room termperature, 2 of 19 mosquitoes showed virus; from lot 136, kept at 30°, 13 of 19 mosquitoes showed virus. This means that 10% of the mosquitoes kept at 24°–27° were infected and 68% of the same mosquitoes kept at 30°. The techniques were, as far as we could tell, identical for the two groups of mosquitoes, except for environmental temperature. That temperature has a prime effect on the length of the extrinsic incubation period is well known (Davis, 1932), but that it should affect the percentage of mosquitoes infected was a complete surprise to us.

To check this, we designed an experiment in which rather extreme conditions would be tested. The mosquitoes that fed on saimiri no. 153 (5th cycle) were divided at random into 3 lots: the main group (lot 153) of 68 mosquitoes was kept at 30°; 22 mosquitoes (lot 154) were kept at a constant temperature of 20°; 22 others (lot 155) were kept for 16 hours every day at 20°, but were transferred for 8 hours (8 A.M. to 4 P.M.) to a constant temperature of 30°. Twenty of the mosquitoes kept at 30° were killed and inoculated in parallel in groups of adult and baby mice at intervals from 10 to 19 days after infection; virus was recovered from 12 (60%). Ten of the mosquitoes kept at 20° were tested in the same way 21 and 22 days after infection; no virus was recovered in any case. Thirteen of the mosquitoes kept at the contrasting temperatures 20°–30° were inoculated from 19 to 22 days after infection, and virus was recovered from 4 (31%). That mere survival of virus in the mosquito is not adversely affected by a temperature of 20° is shown by another lot (142) which was kept at 30° for 18 days and then transferred to 20° for 12 days more. Seven of these mosquitoes were inoculated in mice, and 6 showed virus. Since this temperature rela-

tion is of direct importance in virus epidemiology, more detailed and exact studies are now being undertaken at various constant and alternating temperatures.

Effect of virus dosage

It seems likely that the variation in percentage of mosquitoes showing virus among lots from different transmission cycles kept under the same temperature conditions was due to variation in the amounts of virus ingested. It is difficult to demonstrate this correlation because all titrations of saimiri sera and inoculations of "control" mosquitoes were made in adult mice with consequent irregular results. We have, however, been particularly interested in the possibility of infecting haemagogus on animals circulating small amounts of virus, and several attempts to obtain such infections have been made, in no case with success.

The mosquitoes of lot 117 fed on saimiri 96 (see fig. 2) on the 2nd day after inoculation; the serum of this monkey killed 2 of 6 mice in the 1:10 dilution, 2 of 6 in the 1:100 dilution, and none in greater dilutions. The mosquitoes were kept at room temperature. After 22 days, 12 fed on saimiri 99, which remained normal; at 23 days 7 fed on baby mice, and at 27 days 4 fed on other mice, without transmitting virus. At intervals from 22 to 27 days after the original feeding, 24 mosquitoes were killed and inoculated in adult mice without in any case recovering virus. These mice were tested with a challenge dose of 30 m.l.d. of neurotropic virus, and all succumbed. There was thus no evidence that any of these mosquitoes retained any virus. This experiment was controlled by lot 118, which fed on the same monkey on the 4th day, when the serum killed mice in dilutions up to $1:10^6$; these mosquitoes transmitted to saimiri 103 on the 24th day, and 5 of 15 inoculated in mice showed virus. As we have, in other cases, recovered virus from mosquitoes kept for 10 days at room temperature, the period of 27 days used with lot 117 would seem adequate to allow for a possible slow development of the virus.

A second case is lot 156 which fed on saimiri 153 on the 5th day, when the serum killed mice only in the dilution 1:2; 3 control mosquitoes, inoculated immediately after feeding, gave mouse mortalities of 3/6, 1/5 and 0/6. The mosquitoes were kept for 23 days at 30°, when 6 survivors were killed and inoculated in parallel in groups of adult and baby mice; no virus was recovered. Control for this would be lot 153, which fed on the same monkey on the day previously, when the control mosquitoes gave mortalities of 3/6, 4/5, 5/6 and 5/6; virus was recovered from 60% (12 of 20) of the mosquitoes inoculated after 10 days at 30°.

We have several times attempted to infect haemagogus on mammals other than saimiri in the course of these experiments, always without success, presumably because of the small amount of virus in circulation in the host mammal at the time of feeding. This tends to substantiate the observation published previously (Bates, 1944b) that haemagogus can only be infected on mammals circulating considerable amounts of virus. The evaluation of such negative evidence is difficult, however, and we are continuing experiments.

VI. EPIDEMIOLOGICAL IMPLICATIONS

The field studies of yellow fever that have been carried out in eastern Colombia by Bugher and his co-workers (1944) give a background against which experimental results obtained in the laboratory can be judged. The field evidence implicates *Haemagogus capricornii* as the primary vector of the disease, though it at the same time suggests that other mosquitoes (e.g., *Aedes leucocelaenus*) may at times be important. There is no parallel evidence as to whether a particular mammal species may be dominant in the natural cycles, and most indications are that many species of mammals may play parts in maintaining the virus and that the dominant mammal host, if any, may vary from region to region. There is certainly no evidence that saimiri monkeys are a generally dominant host, and in fact the distribution of yellow fever does not correspond with the distribution of this particular primate, since the disease seems to be endemic in parts of Colombia (especially the Magdalena Valley) where the genus *Saimiri* is unknown. However, it seems sufficiently well established that saimiri monkeys are infected in nature (on the basis of protection test results with wild animals), and the saimiri-haemagogus cycle of virus is thus in all probability a natural cycle, even though it may not be a dominant cycle.

Protection test results are available on 82 saimiri monkeys that were captured in the Villavicencio and Restrepo areas in 1944; most of these were

young animals, not more than a year old. Sera of 10 (12%) gave clearly positive protection test results that can be interpreted as specific evidence of prior infection with yellow fever. Most of these animals came from the region of Restrepo (map in Bugher *et al.*, 1944, p. 18) where there were several known human cases of yellow fever in the course of the year. Other monkeys from the area showed about the same rate of positive protection tests as saimiris (1 of 14 *Callicebus*, and 1 of 8 *Cebus*). Saimiris have not, however, been investigated in direct connection with any known human infection. The only such data available are those given by Bugher *et al.* (1944, p. 45) in which saimiris showed a lower percentage of positives than the other primates examined.

The laboratory evidence indicates that yellow fever infection is highly fatal to saimiris, especially with strains that have been passaged through these animals either by direct inoculation or via mosquitoes. If saimiris were a dominant host in nature, and if this same mortality rate applied, one would expect yellow fever to be a prime cause of death and to result in a great reduction of the monkey population. Yet all of us have the impression that saimiris are the commonest of the local primates. Perhaps the high mortality rate does apply in nature, and perhaps that is the explanation of the relatively low rate of positive protection tests found, most animals not having survived infection. This is the sort of question that could only be answered by quantitative study of the behavior of monkey populations in nature—not a simple task. It is interesting that Laemmert (1944) reports a high mortality rate in Brazilian marmosets inoculated with local strains of yellow fever virus; the laboratory data on susceptibility of marmosets and saimiri monkeys are remarkably similar, and it would seem very likely that their rôles in virus epidemiology in their respective areas of abundance would be equally similar. None of the marmosets is known from the Villavicencio area.

In general, the laboratory and field data in the saimiri-haemagogus cycle complement each other nicely. Saimiri monkeys circulate a high titer of virus (and this is the only local mammal from which we have been able consistently to recover a high titer of virus) and circulating virus of a high titer is apparently necessary for the infection of *Haemagogus capricornii*. The two animals show similar ecological characters: the one is the commonest local primate, the other the commonest local aedine mosquito; both are predominantly arboreal; both are predominantly diurnal; both are common in a wide variety of local forest types (and yellow fever cases show an equally general distribution as regards forest type).

The epidemiological implications of the study of experimental infections in the mosquito are in themselves very interesting. For infection, haemagogus seems to require a relatively high environmental temperature. The species is predominantly arboreal, and day temperatures are appreciably higher in the forest canopy than in the lower forest strata (some data in Bates, 1944c). Shade temperatures of 30°C are, however, very rare in our forests, and the laboratory evidence would seem to indicate that haemagogus, to be an efficient vector, would require higher temperatures than are likely to be found in its habitat. But the mosquito is often remarkably common in sunny situations in the forest, and its habits might well lead resting or active females to spend part of the extrinsic incubation period in sunny canopy situations where the body temperature of the mosquito might well be high enough to be favorable for virus multiplication. The indirect laboratory evidence indicates that haemagogus is remarkably resistant to high temperatures if other conditions are favorable. In individual vials at 30°C, haemagogus has a greater longevity than any of the other local mosquitoes that have been tried, even though these other species, with more conventional laboratory treatment, show a much greater longevity than haemagogus (*e.g.*, in cages at 25°).

This illustrates the well-known difficulty of generalizing from laboratory to natural conditions. We know remarkably little about the longevity of any mosquito species in nature, because of the technical difficulty of obtaining such data. We assume that a vector of quartan malaria must be long lived, because the extrinsic incubation period of the plasmodium in the laboratory is long; we could, by the same reasoning, deduce that *Haemagogus capricornii* must be very long lived in nature; and the deduction is to some extent supported by the long period that adults may be maintained in the laboratory under special conditions. We have the definite impression that haemagogus is physiologically adapted to life at relatively high temperatures and low humidities; these are precisely the conditions of existence in the canopy zone of the tropical forest; and the temperature factor, at least, seems also to be a governing factor in virus infection.

VII. SUMMARY

1. A local strain of yellow fever virus was carried through 5 cycles in the laboratory using saimiri monkeys as hosts and the mosquito *Haemagogus capricornii* as vector.

2. This is a laboratory reproduction of what may be a local transmission mechanism of jungle yellow fever, as both saimiri monkeys and haemagogus mosquitoes have been implicated in the epidemiology of the disease by field studies.

3. The extrinsic incubation period in *Haemagogus capricornii* under the conditions of these experiments was 20 to 24 days at room temperature (24°–27°, mean 25.4°C.); and 13 to 15 days at a constant temperature of 30°C.

4. A virus strain was used that had been subject to a minimum of laboratory manipulation. Adult white mice of the standard strain used in yellow fever studies were found to show an irregular susceptibility to intracerebral inoculation of small amounts of this virus, but 3- and 5-day-old mice were highly susceptible. The strain was highly virulent for saimiri monkeys: 8 of the 10 animals infected by mosquito bite died in the course of acute infections.

5. Only a certain percentage of specimens of *Haemogogus capricornii* became infected after feeding on monkeys with virus in circulation. The percentage infected in a particular experiment appeared to depend on (a) the amount of virus originally ingested by the mosquitoes and (b) the environmental temperature at which the mosquitoes were kept. The possible effect of virus strain and of the genetic constitution of the mosquitoes was not studied.

6. The correlation between factors governing haemagogus infection in the laboratory and the habits of the mosquito judged by field studies, is discussed. The mosquito seems to require a high environmental temperature to become infected, and its habit of flying in the canopy zone of the forest means that it is subject to relatively high temperatures in nature. The correspondence in habits between this species of mosquito and saimiri monkeys in the Villavicencio area is close.

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IMMUNIZATION AGAINST YELLOW FEVER

Studies on the Time of Development and the Duration of Induced Immunity

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The mass immunization of humans in areas of Africa and South America where yellow fever occurs in endemic or epidemic form and of large numbers of persons in the armed forces has brought to the fore the question of the duration of immunity following inoculation with yellow fever vaccine. Moreover the recent extensions of preventive measures and emphasis on their enforcement have made it highly important to know how soon after inoculation persons may be assumed to be immune. Studies designed to throw light on these subjects are reported here.

Our investigations are concerned with the results of immunization with the attenuated yellow fever virus 17D, now widely adopted as standard. This agent was derived from pantropic yellow fever virus by Lloyd, Theiler and Ricci (1) after *in vitro* cultivation first in mouse embryonic tissue, then in chick embryo and finally in chick embryonic tissue from which the nervous elements had been removed. Theiler and Smith (2) made further *in vitro* passages which resulted in marked attenuation of the virus and, after extensive experimental work, used it in human immunization for the first time (3). In monkeys they were unable to demonstrate circulating antibody 7 days after the immunizing injection, but the tests were positive 14 days afterward. However, after virulent inoculation there was no circulation of virus in monkeys vaccinated as many as 7 days before, and all monkeys survived the test inoculation. Thus, although the protection tests were not shown to be positive before the 14th day, the animals exhibited some evidence of active immunity as early as 5 days after vaccination. Unfortunately the control unvaccinated animals in their experiment survived the virulent inoculation, so that the demonstration of immunity in vaccinated animals depended on the tests for circulating virus and on the serum protection tests.

Theiler and Smith (3) also made weekly tests for specific antibody in the serum of 8 persons inoculated with the 17D virus and were able to demonstrate antibody in each of them 2 weeks following their injections. Immunity was not demonstrated 1 week after vaccination.

Smith, Penna and Paoliello (4) reported the first comprehensive field application of the 17D virus. They also failed to demonstrate humoral antibody 1 week after injection and found only 14 of 23 sera protective after 2 weeks. However, in each person tested, antibody was demonstrated 21 days after vaccination. They found that about 95 per cent of vaccinated persons acquire immunity and that the majority retain this immunity for at least 1 year.

Soper and Smith (5) reported that vaccinated humans in yellow fever infected districts escaped infection, whereas non-inoculated members of the same labor gangs contracted the disease. They state that "field experience suggests that the protective effect of vaccination begins not later than a week after inoculation."

In experimental studies of the 17D virus Fox and Penna (6) were able to demonstrate protective antibody as early as 8 days after large doses of vaccine, but found that the antibody developed later when smaller doses were given. They observed that the fully protective reaction of the serum was preceded by an alteration of the protection ratio from the negative to the inconclusive or partially protective range.

With regard to the duration of the immunity induced by antiamaryl vaccination, the most recent comprehensive study is that of Fox and Cabral (7). These authors observed a high incidence of humoral immunity in man as long as 3 or 4 years after vaccination, although there was evidence of a slow decline both in percentage of immunes and in the potency of individual sera. They also ex-

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pressed the opinion that the immune response in children is less satisfactory than in adults.

There are significant differences between the program of immunization in South America and that in Africa, as well as differences in objectives of the postvaccination studies among humans. In Brazil the vaccine has, for the most part, been prepared locally in the laboratory in Rio de Janeiro. Several different substrains of virus have been employed. Administration of the vaccine has been centered in the personnel of the Yellow Fever Service and has been carried out under the direct supervision of physicians expecially trained for this work. The postvaccination studies have been concerned in no small part with determining the relative efficacy of various substrains of virus and with the results obtained by different field units working in different localities under varying conditions.

By contrast, all of the vaccine used in Africa has been prepared and tested for potency in the Laboratories of the International Health Division of The Rockefeller Foundation, in New York, and sent to the laboratory at Entebbe under refrigeration. This has involved an ocean journey of about 11,000 miles, during which the vaccine was kept in ships' refrigerators, followed by a 2-day rail journey during which it was packed in ice. Within Africa the vaccine has been stored and usually shipped under refrigeration but has been released for distribution only after tests in Entebbe have proved its potency. All the vaccine used here has been prepared under a seed-lot system, and differences in substrains are not therefore involved. Field administration of the vaccine has been wholly in the hands of local government medical officers or army officers, only a few of whom have had any special training for this particular work, but all of whom were adequately informed regarding the precautions to be exercised. Postvaccination studies have been primarily concerned with two factors: 1) a determination of the percentage of persons who became immune following inoculation, and 2) a study of the duration of the immunity induced by vaccination. In these studies all of the yellow fever protection tests were done by sensitive methods (8) which employ 1 per cent virus either in adult mice previously prepared by intracerebral injection of starch solution, or in normal 14-day-old mice which have not had starch injection.

EXPERIMENTAL STUDIES

In monkeys. The following experiment was carried out to determine how soon after vaccination protective antibody could be detected in the serum and to ascertain by challenge inoculation the time of appearance of active immunity.

Fourteen unused rhesus monkeys were bled,[3] and yellow fever protection tests were done on their sera. Each was found to be non-immune (table 2). Two were reserved for controls and the other 12 were vaccinated subcutaneously, 1 or 2 per day on 9 different days over a 2-week period, as shown in table 1. The dose of vaccine for each was the standard dose for man, 0.5 ml. of 1 in 10 dilution of rehydrated virus. Each day's inoculum of vaccine was titrated by the intracerebral inoculation of 6 decimal dilutions of vaccine into groups of 12 mice. Titres were determined by the method of Reed and Muench (9). Thus the exact amount of the 17D immunizing virus given to each monkey was determined (table 2).

Fourteen days after vaccination of the first monkey and 1 day after vaccination of the last, each was bled again for protection test and, together with the 2 non-vaccinated controls, inoculated subcutaneously with 1.0 ml. of 1 in 10 dilution of rehydrated pantropic yellow fever virus (Asibi strain from rhesus 24-2). This inoculum was titrated by injecting serial decimal dilutions into groups of 12 mice. The titre of virus was such that each monkey received 121,500 mouse intracerebral MLD of the Asibi virus. Daily thereafter during 10 days, or until death occurred, each of the vaccinated and control monkeys was bled from a vein and its serum was tested for circulating virus (table 1). At the end of the 10 day period each surviving monkey was again bled for protection test.

Results of this experiment are shown in tables 1 and 2. It will be seen (table 2) that all monkeys vaccinated 7 or more days had circulating antibody, while 1 of the 2 vaccinated only 6 days did likewise. The remaining monkey vaccinated 6 days and all those vaccinated less than 6 days failed to exhibit circulating antibody.

One of the unvaccinated monkeys had circulating virus (table 1) daily for 6 days and died on the 6th day; the other had no circulating virus on the first day after the virulent inoculation but did daily

[3] For this and other experimental procedures the animals were anesthetized with ether.

TABLE 1

The response of monkeys to test inoculation of 121,500 mouse MLD of Asibi virus at various intervals after vaccination with 17D virus

RHESUS NO.	INTERVAL BETWEEN VACCINATION AND TEST INOCULATION	MORTALITY RATIOS IN TESTS FOR CIRCULATING VIRUS* Days after inoculation with Asibi virus										FEVER	DIED OR SURVIVED
		1	2	3	4	5	6	7	8	9	10		
	days											*days*	
1	14	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6		S
2	10	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/5	0/6	0/6		S
3	9	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6		S
4	8	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6		S
5	7	0/6	0/7	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6		S
6	7	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	3§	S
7	6	0/6	0/7	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6		S
8	6	0/6	0/7	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6		S
9	5	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6		S
10	5	0/6	0/6	1/6†	0/6	0/6	0/6	0/6	0/6	0/6	0/6		S
11	3	0/6	4/6	6/6	6/6	6/6	0/6	0/6	0/6	0/6	0/6	4, 5, 6	S
12	1	6/6	7/7	6/6	6/6	6/6‡						3, 4	D
13	Not vaccinated	0/6	6/6	6/6	6/6	6/6‡						3, 4	D
14	Not vaccinated	2/6	7/7	6/6	6/6	6/6	6/6‡					3, 4, 5	D

* Numerator indicates number of mice which died, denominator the number inoculated.

† Death of this mouse was proved to be due to yellow fever virus.

‡ Animal died on this day.

§ 104.0°F. in p.m. only. Normal temperature of this monkey: 103.0–103.6°.

TABLE 2

Yellow fever protection tests on sera of monkeys before and at various intervals after vaccination with 17D virus, showing the time of appearance of demonstrable antibody

RHESUS NO.	MLD VACCINE INJECTED	INTERVAL BETWEEN VACCINATION AND TEST INOCULATION	SERUM PROTECTION RATIO*		
			Before vaccination	After vaccination†	10 days after Asibi
		days			
1	5293	14	0/6	6/6	6/6
2	8016	10	0/6	6/6	6/6
3	3841	9	1/6	6/6	6/6
4	7869	8	0/6	5/6	6/6
5	10521	7	1/6	5/6	6/6
6	10521	7	0/6	5/6	6/6
7	6513	6	1/6	5/6	6/6
8	6513	6	0/6	1/6	6/6
9	7869	5	0/6	0/6	6/6
10	7869	5	0/6	0/6	6/6
11	13861	3	0/6	1/6	6/6
12	25885	1	0/6	0/6	
13	0	Not vaccinated		0/6	
14	0	Not vaccinated		0/6	

* Numerator indicates number of mice which survived, denominator the number inoculated.

† Immediately before Asibi inoculation at stated interval after vaccination.

thereafter for 4 days, and succumbed on the 5th day. The monkey vaccinated 1 day prior to test inoculation had circulating virus daily for 5 days and died on the 5th day. Gross and microscopic lesions in these 3 animals were typical of yellow fever. The monkey vaccinated 3 days prior to inoculation had circulating virus on the 2nd to 5th days and became severely ill, but recovered. One of the monkeys vaccinated 5 days had the minutest trace of circulating virus[4] on the third day after inoculation of Asibi virus, but none on other days, and survived the infection without even having fever (table 1). The other monkey vaccinated 5 days, and all vaccinated more than 5 days, failed to show any circulating virus or other ill effects following inoculation with the test virus.

Each of the 11 surviving monkeys had protective antibody in its serum 10 days after the challenge injection (table 2).

Thus, immunity as indicated by protective action of the serum, was demonstrable 6 or 7 days after vaccination; but monkeys were fully resistant to infection 5 days after inoculation. This apparent lag in appearance of antibody may have been due to enhancement of antibody titre during the

[4] The serum caused the death of only 1 of 6 mice, but passage and a protection test proved that death was due to yellow fever virus.

incubation period of the disease, or it may be that other factors associated with immunity antedate the appearance of demonstrable antibody. However this may be, the results confirm and extend the observations of Theiler and Smith (3) and demonstrate the early appearance of an effectual protective mechanism following yellow fever vaccination.

In man. The following experiment was done to ascertain how soon after vaccination circulating antibody could be demonstrated in man. The essential difference between this experiment and similar ones by others was our use of a more sensitive method (8) for detecting the antibody.

TABLE 3

Yellow fever protection tests on the sera of humans before and at various intervals after inoculation with standard yellow fever vaccine virus 17D

VOLUNTEER NO.	PROTECTION RATIO*				
	Before vaccination	Days after vaccination			
		7	10	14	21
1	0/6	1/6	6/6	6/6	6/6
2	0/6	4/5	6/6	6/6	6/6
3	0/6	0/6	4/6, 8/8†	6/6	6/6
4	0/6	2/6	6/6	5/5	6/6
5	0/6	0/6	4/6, 7/8	6/6	6/6
6	0/6	0/6	0/6	2/6, 8/8	5/6
7	0/6	0/5	3/6, 7/8	6/6	6/6
8	0/6	1/5	5/6	5/6	6/6
9	0/6	1/6	5/6	6/6	5/5
10	0/6	0/6	4/6, 8/8	6/6	6/6

* Numerator indicates the number of mice which survived, denominator the number inoculated.

† 8/8 and 7/8 are results of highly sensitive tests in 14-day-old mice, all other tests being done in mice 35 days old.

Ten adult male African volunteers, each non-immune to yellow fever, were inoculated subcutaneously with 0.5 ml. each of a 1 in 10 dilution of 17D vaccine virus, lot 1110-1, and the vaccine was titrated as in the previous experiment. The potency of the vaccine was such that each man received 10,000 mouse intracerebral MLD. At intervals of 7, 10, 14 and 21 days after vaccination, each man was bled for protection test, all the post-vaccination sera being tested in the same test run. Table 3 shows the results of these tests.

From the data in table 3 it will be seen that 1 of the 10 men had circulating antibody on the 7th day, 9 of 10 on the 10th day, and the sera of all were protective by the 14th day. Thus, antibody was not demonstrable in man quite so early as in rhesus monkeys but there was a very high incidence of humoral immunity by the 10th postvaccination day. Judging from the inoculation experiments in monkeys it seems highly probable that an effectual protective mechanism against infection would be operative at least by the 8th or 9th day.

POSTVACCINATION IMMUNITY SURVEYS

Per cent of humans immunized by the inoculation. Persons inducted into the armed forces in East Africa in recent years have been inoculated against yellow fever with 17D vaccine supplied by this Institute. Inoculation centers for such personnel have been established in many places throughout East Africa and the inoculations have been done by various officers, none of whom were specially trained for this purpose. The centers in some instances are not readily accessible, and it was deemed desirable to check on the results obtained under active service conditions. Representative samples of personnel vaccinated in numerous centers were obtainable at 2 depots in East Africa through the courtesy of Brigadier R. P. Cormack, Director of Medical Services, East Africa Command. Samples of blood were taken from 103 persons whose pay-book records showed the date and place of vaccination. The greater proportion of these persons came from areas where yellow fever is not known to have occurred and where protection test surveys are negative. The sample included individuals who had been vaccinated at 29 different inoculation centers as widely separated as Jinja, Uganda; Lusaka, N. Rhodesia; Zomba, Nyasaland; and Ceylon. Sixty-two of the persons bled had been inoculated less than 6 months, 26 had been inoculated more than 6 months but not more than 1 year, while the remaining 15 had been inoculated 13 to 22 months.

When these specimens were examined in the yellow fever protection test one gave an inconclusive result[5] in 3 tests and had to be excluded. Ninety-four, or 92.2 per cent, were found to be protective, and 8 were non-protective. The negative sera came from individuals vaccinated in several different localities, and there was no indication that impotent vaccine or faulty technique had

[5] This man had been inoculated only 13 days before his blood was taken and it is probable that his serum antibody titre was rising.

been employed anywhere. Results are shown in table 4.

This result was regarded as quite satisfactory, since a great variety of modes of transportation for the vaccine were involved, as well as a diversity of local conditions of supply and climate and the inevitable variations of the "human factor." It showed that, notwithstanding all the distance covered between the production laboratory and the recipient of the vaccine, and the time elapsed, a very high per cent of persons become immune as result of the inoculation.

Duration of the immunity induced by vaccination. Two zones in British East Africa are admirably suited to investigation of the duration of immunity induced by vaccination: the Kenya coastal belt and the Toro district in western Uganda.

Early in 1941 a program of mass vaccination against yellow fever was undertaken in the Kenya coastal belt, principally directed toward preventing the eastward spread of the disease. All the residents and the non-resident laborers in a 10-mile-wide area along the Kenya shore of the Indian Ocean were inoculated, the program being under the direct supervision of Dr. C. R. Philip of the Kenya Medical Service. A certificate bearing the date of inoculation and the lot of vaccine used was issued to each person injected. In all, about 335,000 persons were inoculated in this area between April 1941 and May 1942 with 17D vaccine supplied by this Institute. Between November 1943 and April 1944, 181 of these persons were bled by Dr. James M. Liston, Kenya Medical Service, and the specimens were sent to this laboratory for testing. Through the vigilance of Dr. Liston, only persons believed to be the rightful possessors of the certificates presented were bled. Candidates of various ages were selected. Also, persons vaccinated on different days, at different places and with different lots of vaccine were sampled, the sera including specimens from individuals inoculated in at least 14 different places (place not stated for 4 donors) with 13 different batches of vaccine. The donors were bled 23 to 36 months after receiving the vaccine, the mean interval being 30 months. No lot of vaccine was responsible for a significantly high per cent of failures, and no inoculation center was found to have obtained poor results.

TABLE 4

Summary of postvaccination yellow fever protection tests on military personnel and on residents of Kenya and Uganda

SOURCE OF SERA	INTERVAL BETWEEN VACCINATION AND TESTS	NO. TESTED	NO. IMMUNE	PER CENT IMMUNE	AGE GROUP	NO. TESTED	NO. IMMUNE	PER CENT IMMUNE
	months							
Army	1–22 mean 6.8	102*	94	92.2	Adults	102	94	92.2
Kenya coastal area	23–36 mean 30	170†	153	90.0	Children‡ Adults	24 146	23 130	95.8 89.0
Bwamba County, Uganda	24	300	278	92.7	Children Adults	139 161	128 150	92.1 93.2
Bwamba County, Uganda	36	300	280	93.3	Children Adults	150 150	141 139	94.0 92.7

* One other which gave an inconclusive result is excluded.

† Three others which gave inconclusive results and 8 which were toxic are excluded.

‡ Children include persons 0–14 years of age, adults include persons 15 years old and over.

Results of the Kenya survey are shown in table 4. Eleven of the specimens had to be eliminated from consideration because they gave toxic (8 specimens) or inconclusive (3 specimens) results. Of the remaining 170 specimens, 153 (just 90 per cent) were protective. From the results shown in table 4 it is clear that the children retained their immunity quite as well as the adults.

At about the same time as the mass vaccination program in the Kenya coastal area, a similar program was undertaken in the Toro district of western Uganda. This was done in order to arrest

locally an outbreak of yellow fever then in progress in Bwamba County and to prevent its spread afield (10). Between May 1941 and August of that year 145,152 persons were inoculated with 17D vaccine in this district, under the supervision of Dr. J. C. St. G. Earl, then Senior Medical Officer, and Dr. J. K. Hunter, District Medical Officer, both of the Uganda Medical Service. A determined effort was made to have every person in the demarcated area inoculated, from babes at breast to the eldest residents. Persons unable to walk were carried to the inoculation centers.

different batches of vaccine. The population of the county is estimated at 35,044, so that the sample included nearly 1 per cent of the whole.

Sampling in this area was done wholly without reference to certificates or statements of the donors regarding inoculations, as it had already been found (10) in this same region that the only reliable means of identifying any given person for any purpose was by finger-printing, a procedure not possible in this instance. After collection of each sample a notation was made regarding the donor's statement that he had or had not been vaccinated, but in the

TABLE 5

Postvaccination yellow fever protection tests on sera of residents of Bwamba County, Uganda, showing results for different age groups in various areas within the County

AREA	ESTIMATED POPULATION	AGE GROUP	2 YEAR SURVEY			3 YEAR SURVEY		
			Number examined	Number immune	Percentage immune	Number examined	Number immune	Percentage immune
Busaro	8705	0–14	35	33	94.3	35	34	97.1
		15 and over	35	35	100.0	35	32	91.4
Bubandi	7705	0–14	30	28	93.3	30	26	86.7
		15 and over	30	27	90.0	30	28	93.3
Hakitengya	8499	0–14	35	32	91.4	35	35	100.0
		15 and over	35	35	100.0	35	33	94.3
Buhundu	7233	0–14	20	17	85.0	30	28	93.3
		15 and over	40	33	82.5	30	29	96.7
Rwebisenge	2902	0–14	19	18	94.7	20	18	90.0
		15 and over	21	20	95.2	20	17	85.0
Total	35,044	0–14	139	128	92.1	150	141	94.0
		15 and over	161	150	93.2	150	139	92.7
Grand total			300	278	92.7	300	280	93.3

Extensive yellow fever investigations had already been made in a portion of the Toro district (10, 11, 12), namely Bwamba County, and the postvaccination survey was carried out there. The county is divided for administrative purposes into 5 gombololas, each with its chief. These in turn are subdivided into varying numbers of local units called murukas, each with its local chief. There are, in all, 30 murukas in Bwamba. The postvaccination sample included blood specimens from 10 persons, usually 5 adults and 5 children, from each muruka, or a toll of 300 specimens. Since the vaccinations were done by administrative units this gave a representative sample of persons inoculated at different places and times, and with

final analysis these statements proved to be of no significance. Children born after the completion of the vaccination program were rejected as being known non-vaccinated individuals.

Postvaccination surveys of immunity in Bwamba were made just 2 years and again just 3 years after the mass inoculations. A summary of the results of both surveys is shown in table 4 along with the results from the Service group and the survey of the Kenya coast. To study further the results in Bwamba, table 5 has been prepared showing the findings of the 2 and 3 year surveys by regional localities.

The results in table 5 have a significance which is not apparent from the data shown. It has already

been stated that the distribution of immunity to yellow fever in Bwamba prior to the mass inoculations was not homogeneous (10, 11, 12). Surveys made in 1937–1940 showed that 10.8 per cent of the general population was immune (12) and that in the middle, forested portion the incidence (20 per cent) was more than twice as high[6] as in the southern grasslands area (8 per cent) (10). Moreover, in many localities in the grassland area (Bubandi Gombolola) and on the slopes of the Ruwenzori mountains (Buhundu Gombolola) there was a complete absence of immunity in children. After the mass inoculations, however, the incidence of immunity was of quite even distribution throughout the County and the rate in children was as high as that in adults (table 5). Furthermore, 3 years after completion of the vaccination program the children retained their immunity as well as the adults.

Thus in each of 3 population groups of significant size, 90 per cent or more of persons were found to be immune following inoculation with yellow fever vaccine 17D, in tests done as long as 3 years after the vaccination. There was no decline in the immunity rate during the third year, as shown by comparing the 2 year and the 3 year results from Bwamba County, Uganda. The results are in agreement with those reported recently by Bugher and Gast-Galvis (13) from Colombia. They speak well for the use of the vaccine in general practice, so to speak, and indicate that the validity of the yellow fever vaccination certificate may safely be extended well beyond 3 years without regard to the age of the vaccinated person.

Plans have been laid for the repetition of these surveys in the future to obtain more information on the duration of the immunity induced by vaccination.

SUMMARY

1. Protective antibody against yellow fever virus is demonstrable in the serum of rhesus monkeys within 6 or 7 days after inoculation with standard 17D yellow fever vaccine virus.

2. Rhesus monkeys are completely resistant to the inoculation of highly virulent pantropic yellow fever virus within 5 or 6 days after injection of 17D vaccine. This resistance is present prior to the appearance in the serum of demonstrable protective antibody.

3. Protective antibody is demonstrable in man in a high per cent of cases by the 10th day after injection of 17D vaccine and may be present as early as the 7th day.

4. Postvaccination surveys of immunity were made in persons inoculated in Africa with 17D vaccine prepared in New York, and revealed the following:

a. 92.2 per cent of military personnel sampled 1 to 22 months after vaccination exhibited protective antibody.

b. 90 per cent of civilians inoculated in Kenya exhibited protective antibody in their sera 23 to 36 months after receiving the vaccine.

c. More than 90 per cent of persons vaccinated in Uganda had protective sera after 3 years, and there was no decline in the incidence of immunity during the third year.

d. The percentage of children who became immune as the result of inoculation was as great as that in adults, and the antibody response was equally well maintained.

[6] The incidence of immunity to yellow fever in this forested region was further elevated to more than 40 per cent by an outbreak of the disease which occurred early in 1941 (10), this epidemic being the occasion for the mass inoculations which followed.

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STUDIES ON CYCLIC PASSAGE OF YELLOW FEVER VIRUS IN SOUTH AMERICAN MAMMALS AND MOSQUITOES[1]

MARMOSETS (CALLITHRIX AURITA) AND CEBUS MONKEYS (CEBUS VERSUTUS) IN COMBINATION WITH AEDES AEGYPTI AND HAEMAGOGUS EQUINUS

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INTRODUCTION

In efforts to unravel the epidemiology of jungle yellow fever and determine the manner in which the virus is maintained in certain forested areas of South America, many species of indigenous vertebrates and arthropods have been investigated as possible hosts and vectors.

Susceptibility of vertebrates has usually been gauged by inoculating graded doses of virus or, less frequently, by infecting through the bite of a mosquito and subsequently testing for the amount and duration of the circulating virus. Only occasionally has cyclic transmission by means of alternate passage through insect vector and vertebrate host been attempted in animals native to the forests where the virus has been found, and in no instance has the virus been maintained in them in combination with a vector beyond one complete cycle. Likewise, experimental evidence incriminating sylvan mosquitoes as vectors is based upon single rather than serial cyclic transmissions.

While such experiments constitute the first logical step and have yielded much valuable information, they are not entirely satisfying to the epidemiologist. The mere fact that an animal circulates virus following artificial introduction of the virus does not necessarily imply that the animal may be a potential natural host. Nor does one single vector-animal-vector cycle furnish much additional proof. The epidemiologist would like to know whether a given vertebrate or insect can serve in the *continuous* cyclic passage of the virus.

If an animal does play an essential part in the maintenance of the virus under natural conditions it should be possible to reproduce these conditions in the laboratory and thus maintain the virus at will through repeated alternate animal-vector cycles. Conversely, it may be argued that unless an animal fulfills this host requirement in combination with a suitable vector, it is improbable that it alone can be the host responsible for the maintenance of the virus in nature.

It would seem justifiable to apply a similar criterion to suspected arthropod vectors. That is, an effective vector should be capable of transmitting the virus in series in combination with a suitable vertebrate host. Experiments were therefore devised to determine the efficacy of vertebrate hosts on the one hand, and, on the other, the efficacy of insect vectors, in maintaining the virus in serial cyclic transmissions.

Since primates, captured following the passage of jungle yellow fever through a region, have frequently been found immune, and since they are in general rather highly susceptible to laboratory infection with the virus, a species from each of the two widely distributed genera of primates in Brazil was first chosen for study. The species were *Callithrix aurita*, belonging to the marmoset or *Callithrix* genus, and *Cebus versutus*, belonging to the *Cebus* genus of monkeys.

Davis (1, 2, 3), to whom we are indebted for extensive studies on the susceptibility of Brazilian primates to yellow fever, found that three species of the *Cebus* monkey, *macrocephalus*, *albifrons*, and *frontatus*, and two species of marmosets, *Callithrix albicollis* and *Leontocebus ursulus*, could be infected not only by inoculation of infectious material but also by the bite of mosquitoes which had previously fed on infected rhesus monkeys. He also demonstrated that the virus could be transferred back to rhesus monkeys through mosquitoes that had fed on infected cebus monkeys and the two species of marmosets. He did not attempt,

[1] The work on which these observations are based was done under the auspices of the Serviço de Estudos e Pesquisas sôbre a Febre Amarela (Yellow Fever Research Service), which is maintained jointly by the Ministry of Education and Health of Brazil, and the International Health Division of The Rockefeller Foundation.

however, to continue the alternate host-vector transmissions beyond one complete cycle in *Callithrix albicollis*. *Aedes aegypti* was employed as the insect vector. It may be added that at the time these experiments were performed, it had not been proved that the virus may be harbored in forested areas independent of the human-aegypti cycle.

Laemmert (4) has since confirmed and extended the observations of Davis on the high susceptibility of marmosets. He also showed that the marmoset may infect and be infected by mosquitoes, but again the mosquito-marmoset transmission was not pursued beyond one cycle.

Since yellow fever virus has been repeatedly isolated from wild caught haemagogus mosquitoes, it seemed indicated to investigate further the vector efficiency of this genus (5, 6, 7, 8). Experimental transmissions with haemagogus mosquitoes have been recorded, but the results have not been consistent (9, 10). The experiments here reported on cyclic transmissions with a species of this genus are incomplete. However, they have been included, as they furnish some additional information on its efficacy as a vector.

MATERIALS AND METHODS

Virus strains. Two Brazilian and one Colombian strain of jungle yellow fever virus were employed. The Brazilian strains, Olympio Christo (O.C.) and João Zanabrea (J.Z.), have been described by Laemmert (4). Both were isolated from patients with non-fatal cases of jungle yellow fever by the inoculation into rhesus monkeys of blood withdrawn from the patients during the acute stage of the disease. In these experiments the O.C. strain was used in its seventh consecutive rhesus monkey passage, and the J.Z. in its sixth. The Colombian Volcanes strain was isolated in 1943 from a rhesus monkey bitten by haemagogus mosquitoes captured in the forests of Volcanes, Colombia. The desiccated serum[2] from this first passage rhesus monkey was used to initiate the cebus monkey-mosquito cycles with this virus strain.

Although none of the strains had been passed previously through mice, all were found highly infective to these animals and could be satisfactorily titrated by intracerebral inoculation.

Vertebrate hosts. The marmosets (*Callithrix aurita*[3] E. Geoff) were purchased from a local trapper at Guaratiba, State of Rio de Janeiro. None of them possessed neutralizing antibodies for yellow fever virus when captured, and, as far as is known, the virus was never found in the region whence these animals came. This particular species, while closely related to other members of this very widely distributed genus, has a habitat limited to the littoral of the states of Rio de Janeiro and São Paulo.

The cebus monkeys (*Cebus versutus*[3] Elliott) were captured in the region of Araguarí and Uberaba, State of Minas Gerais. A wave of jungle yellow fever passed through this region about six years previously, and a few of the older animals possessed neutralizing antibodies to yellow fever virus. None of the positive reactors were used in the experiments. Monkeys of this species, or ones very similar, are found in forests over a vast area extending northward from Argentina through the entire expanse of Brazil.

Insect vectors. *Aedes aegypti* Linnaeus were used in the experiments designed to test the efficacy of cebus monkeys and marmosets as hosts of yellow fever virus, because they are known to be good vectors and also because they were the only mosquitoes constantly available in adequate numbers. All of the aegypti came from a laboratory colony established in 1942 from females captured at Nova Iguassú, State of Rio de Janeiro.

Later, laboratory reared *Haemagogus equinus* Theobald were employed in conjunction with marmosets. The colony stemmed from eggs obtained from Osorno-Mesa (11), who had previously succeeded in colonizing this mosquito in Bogotá, Colombia.

The mosquitoes were infected and stored essentially as described by Stokes, Bauer, and Hudson (12). The *H. equinus* were reared, and maintained following infection, at a more or less constant temperature (26–28°C) and humidity (R.H. 70–90 per cent). Some of the *A. aegypti* were kept in the air-conditioned room following infection, but most of them were subjected to the usual laboratory environment (ranging in temperature from 20–34°C).

[2] The desiccated serum was kindly sent to us by the Laboratory of the Section of Special Studies, maintained by the Ministry of Labor, Hygiene, and Social Welfare of the Republic of Colombia and the International Health Division of The Rockefeller Foundation.

[3] We are indebted to Dr. J. Moojen of the Museu Nacional in Rio de Janeiro for making these indentifications and furnishing information on habitat.

Virus determinations. In order to verify transmission of infection to the host and ascertain whether the next passage mosquitoes had ingested virus, either serum from blood withdrawn from the animal immediately after the mosquitoes had fed or an emulsion of five to ten engorged mosquitoes was inoculated intracerebrally into six white mice. When quantitative determinations were desired, serial tenfold dilutions of serum or of mosquito emulsion were inoculated intracerebrally into groups of six mice for each dilution. All dilutions, as well as the initial suspension of the triturated mosquitoes, were made in 0.85 per cent NaCl containing 10 per cent of normal human serum.

In addition, mosquitoes were occasionally tested for virus following a suitable incubation period, by permitting them to bite two-day-old mice or by intracerebral inoculation of an emulsion of triturated mosquitoes into adult mice. This procedure was not followed consistently, as the continuation of the cycle and the ability of the mosquitoes to transmit the virus was determined by whether or not the next passage animal became infected.

Tests for immunity. Only animals giving a negative neutralization test for yellow fever virus were used in these experiments. Blood was withdrawn (from the survivors) 21 days following exposure to the test mosquitoes, and in order to facilitate comparison this sample was tested simultaneously with the pre-exposure sample. The intracerebral technique described by Theiler (13) and modified by Bugher (14) was employed. The mice were between 21 and 28 days of age, and the unit of virus approximated 100 MLD.

Technique employed in host-vector cycles. The cycles were initiated in the host either by subcutaneous inoculation of the virus or by the bite of known infectious mosquitoes. At intervals thereafter, ranging from the second to the seventh day, batches of mosquitoes were permitted to feed, it having been previously determined that the virus usually circulated in marmosets and cebus monkeys within this period. The feedings were accomplished by strapping the animal back-downward on a padded board, removing the hair from a portion of the abdomen, and introducing the immobilized animal into the cage containing the mosquitoes. At the end of one-half to one hour, depending upon the avidity with which the mosquitoes fed, the animal was withdrawn. The insects which had not fed were culled and discarded. Those which had ingested blood were stored until the presence or absence of circulating virus at the time of the blood meal was determined. If no virus could be demonstrated the mosquitoes were discarded.

The lots which had ingested virus were pooled. Following adequate incubation periods, the pooled insects were permitted to feed on one or more normal animals and thus the host-vector-host cycle was continued. The number of mosquitoes used in making the transfers varied considerably, as it was dependent upon three unpredictable factors: the percentage of the exposed mosquitoes taking the infective meal, the number surviving the incubation period, and the percentage feeding on the next passage animal.

EXPERIMENTAL

Callithrix aurita–Aedes aegypti series. This series was commenced by inoculating a marmoset subcutaneously with rehydrated serum from a rhesus monkey infected with O.C. virus. Mosquitoes were fed on this marmoset when the virus titer in the blood was high, and 28 days later the mosquitoes were permitted to bite a second marmoset. From then on the host-vector-host cycles were continued through the ninth cycle according to the technique previously described. The results are schematically recorded in fig. 1.

In no instance did an exposed marmoset fail to become infected. All showed virus in the blood when first tested on the second or third day. Fifteen of the 17 exposed animals died on the fourth to the eighth day. Virus continued to circulate until death. One of the two that recovered circulated virus only up to the fourth day, the other up to the fifth day. Both were immune when tested on the twenty-first day following exposure to the infected mosquitoes.

The number of days elapsing between the infective meal of the mosquitoes and the time at which they were permitted to feed upon the next normal animal in the series varied from 19 to 73 days. The average incubation period was 34 days.

The number of mosquitoes used in making the passages ranged from four to 120 with an average of 47. In only five instances were the mosquitoes otherwise tested (in mice) for the presence of virus. In each instance virus was demonstrated.

It was the plan to discontinue passages after five cycles had been achieved, as it was believed this number should suffice to demonstrate that,

barring accident, the cycles could be continued indefinitely. In this instance the passages were prolonged as a source of infectious mosquitoes for other experiments. Two or more animals were sometimes used in the passages but, considering the consistency with which mosquitoes transfer this virus strain from one marmoset to another, only one passage animal should be required for keeping the cycle chain intact. Nor does there appear to be any diminution in the severity of the infection. The last marmoset recorded in the series died from the infection and

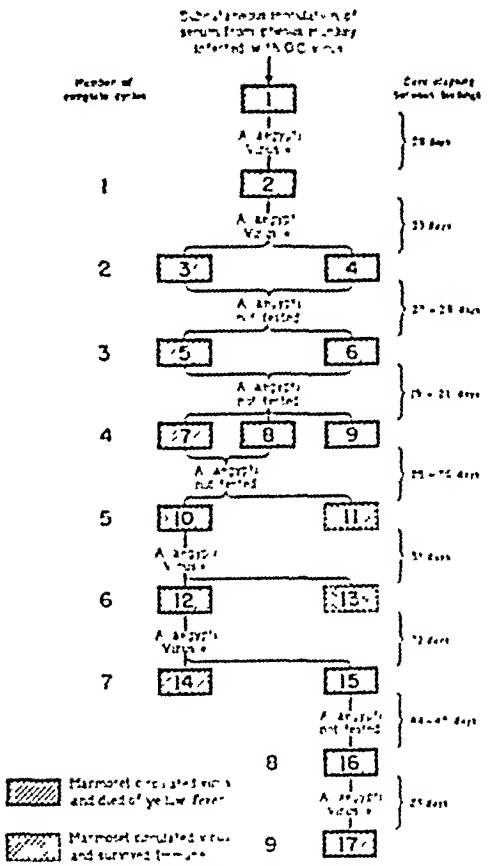

FIG. 1

the serum was infectious to mice in a dilution exceeding 10^{-7}.

Cebus monkey (Cebus versutus)–Aedes aegypti cycles. Aedes aegypti which had been infected 42 days earlier on a rhesus monkey inoculated with the J.Z. virus strain were allowed to bite a pair of normal cebus monkeys. Fresh lots of mosquitoes were fed on both animals from the third to the seventh day following exposure to the infected mosquitoes. After an incubation period of 36 to 40 days, a pool of the mosquitoes that had fed at a time when virus was circulating, was permitted to bite two other normal cebus monkeys. By following this general procedure it was possible to maintain the virus through five complete cycles. The series was then voluntarily discontinued.

All of the exposed cebus monkeys circulated virus and those that recovered developed immunity (fig. 2). Only two of the 13 used in the experiment died, and one of them died from a cause other than yellow fever. Five of the six pools of mosquitoes used in transferring infection were tested for virus either by feeding them upon baby

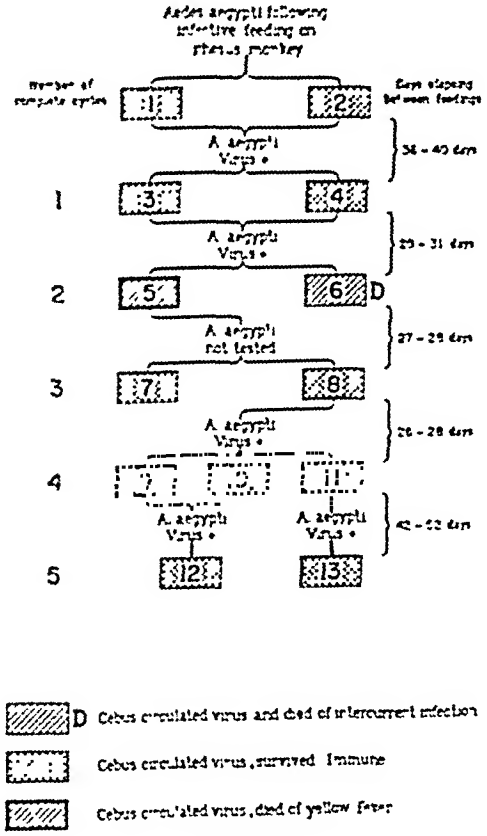

FIG. 2

mice or by intracerebral inoculation of a suspension of the triturated mosquitoes. All of those tested were found to contain virus. The periods of incubation of the mosquitoes between the infective and the test feeding varied from 26 to 52 days and the number of mosquitoes used for passages ranged from 15 to 143. The mean was 68.

Titration of circulating virus in cebus monkeys used in this as well as other experiments indicates that the virus is invariably present during the the third, fourth, and fifth postinfection days, is usually present on the sixth, and in five of

eight monkeys tested was present on the seventh day. However, the concentration is low when compared with that found in the marmoset. The maximum titer may not exceed 10^{-4} and rarely reaches 10^{-6}.

The J.Z. strain was used in the above-mentioned experiment because it seemed to be more virulent for cebus monkeys than most other jungle strains. Having been successful in maintaining this relatively virulent strain, it was decided to use one of the least virulent of the jungle strains then in our possession, the Volcanes strain from Colombia.

Two successful cycles were completed with this strain. The experiment was abruptly terminated by the accidental exposure of the third cycle mosquitoes to insecticide. The lone survivor was found to contain an abundance of virus when triturated and inoculated intracerebrally into mice. However, not all of the exposed monkeys circulated this virus strain in concentration adequate to infect mosquitoes consistently. It would appear, therefore, that in order to assure continuous propagation of this relatively avirulent strain in cebus monkeys several animals may be required for each passage.

Callithrix aurita–Haemagogus equinus series. It having been shown that the marmoset (*Callithrix aurita*) is a suitable animal for consistently infecting batches of *Aedes aegypti*, the next step was to determine whether *Haemagogus sp.*, a sylvan mosquito, could likewise serve as a vector in combination with this proven good host. While there is no great difficulty in rearing *H. equinus* under captive conditions, the females tend to die off rather rapidly. At least this has been our experience. The high mortality among the mosquitoes following the infective feeding introduced complications not encountered with the domestic *A. aegypti* and necessitated considerable reduction of the incubation periods or the number of mosquitoes used in making the passages. The experiments here reported are incomplete but nevertheless throw some light on the efficacy of this species as a vector of yellow fever virus.

The cycles were inititated by feeding *H. equinus* upon a marmoset from the seventh aegypti-marmoset cycle. On the 26th and the 27th day following feeding the two survivors were allowed to bite a normal marmoset. The transmissions from here on are graphically presented in figure 3. Three cycles were completed when the series was interrupted owing to the lack of fresh equinus females to feed on the third passage marmoset prior to its death from yellow fever virus infection.

In interpreting the successful as well as the unsuccessful attempts at transmission in this series consideration should be given to both the number of mosquitoes involved and the period elapsing between the infective and the test feedings. In two of the three failures to transmit, the mosquitoes were used from 7 to 10 and from 13 to 15 days following feeding on the infected animal. These periods are dangerously near the minimum incubation time required for a mosquito to transfer the virus by bite. Although the time interval was adequate in the third unsuccessful attempt, only one mosquito was involved. On the other hand, the four successful transfers from

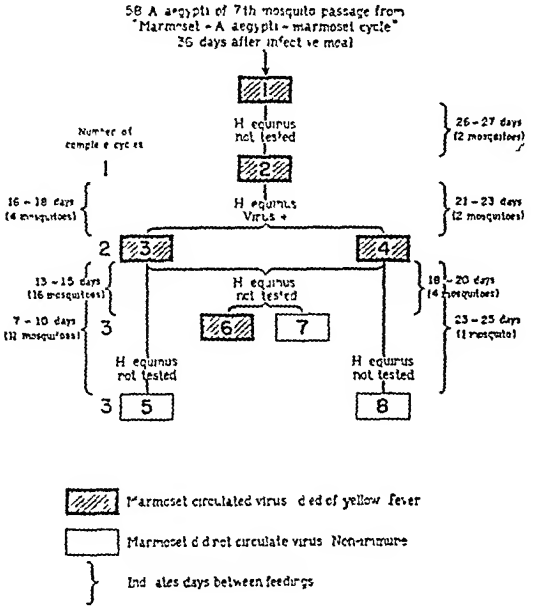

FIG. 3

marmoset No. 1 to No. 2, from No. 2 to No. 3 and to No. 4, and from Nos. 3 and 4 to No. 7, were made with two, four, two, and four mosquitoes respectively. Indeed, under similar conditions of shortened incubation period and the small number of mosquitoes employed, it is doubtful if the record of *Aedes aegypti* would have been any better.

It may be added that in a single experiment three *H. spegazzinii*, a species found in regions where jungle yellow fever is endemic in Brazil, were able to transmit the virus from an infected to a normal marmoset. Owing to lack of mosquitoes the cycle was not continued.

DISCUSSION

It has been shown that in combination with a suitable insect vector certain strains of jungle

yellow fever may be maintained at will in cyclic passages through marmosets (*Callithrix aurita*) or cebus monkeys (*Cebus versutus*). While most of the passages were made in conjunction with *Aedes aegypti*, a mosquito which admittedly plays no part in the epidemiology of jungle yellow fever, some preliminary experiments imply that certain members of the genus *Haemagogus* may serve equally well as vectors of the virus.

It is quite conceivable that, given an adequate population of marmosets or cebus monkeys in association with an effective insect vector, yellow fever virus may be harbored in forested areas, probably not for a long period in any one locality but rather in the form of wandering epidemics. Indeed, epidemiological observations, even in regions where the disease is "endemic," suggest that the virus does not remain long at any one place (6, 7, 8). It tends to "burn out" and migrate from one locality to another as conditions favor.

Inasmuch as the period of circulation of the virus in both marmosets and cebus monkeys is usually limited to a few days, the mosquito may, from the aspect of time, be considered more of a reservoir of the virus than the animal host, since the mosquito once infected commonly remains infectious throughout its entire life (8, 12). This feature has been remarked upon by Bugher and Boshell (6).

While it is believed that these experiments lend additional support to the concept that the virus may be preserved in forested areas by alternate passage through sylvan mosquitoes and South American primates, they do not preclude the possibility that some other vertebrates or arthropods may play a role in the epidemiology of jungle yellow fever.

Similar studies with marsupials, other species of marmosets, and mosquitoes of the *Haemagogus* genus are now in progress.

SUMMARY

A strain of yellow fever virus of jungle origin was easily maintained by alternate passage through a species of marmoset (*Callithrix aurita*) and *Aedes aegypti* mosquitoes. Nine cycles have been completed.

Yellow fever virus, also of jungle origin, was similarly passed through cebus monkeys (*Cebus versutus*) and *Aedes aegypti*. Five cycles were completed when the series was voluntarily interrupted.

Using *Haemagogus equinus* as vector in conjunction with the marmoset as host, three cycles were completed before the series was interrupted for lack of fresh mosquitoes.

The implication of these observations in relation to the epidemiology of South American jungle yellow fever is briefly discussed.

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THE ISOLATION OF YELLOW FEVER VIRUS FROM WILD-CAUGHT MARMOSETS[1]

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INTRODUCTION

It has been presumed by investigators of jungle yellow fever that virus may be preserved in certain forested areas by cyclic transmission through vertebrate hosts and insect vectors. Suspicion has fallen upon members of the order of primates, since they are susceptible to experimental infection and have frequently been found immune to yellow fever in regions where the disease is endemic and also in regions through which an epidemic wave has passed. However, yellow fever virus has never been isolated previously from naturally infected vertebrates other than man.

The object of the present communication is to report the isolation of yellow fever virus from captured marmosets on four separate occasions. These isolations were made during the course of epidemiological investigations in the vicinity of Ilhéus, State of Bahia, where, presumably, the disease is endemic. The study included extensive capture of wild animals, principally for the purpose of determining the incidence of specific yellow fever immunity, but also with the object of demonstrating the presence of virus.

Isolation of virus. In the area selected for epidemiological studies marmosets of the species *Callithrix penicillata* (E. Geoffrey, 1812) are quite plentiful, and 1,437 marmosets of this species have been captured or purchased from local trappers. Early in the investigation results of neutralization tests showed that a significant proportion of marmosets captured in certain areas in the general region possessed yellow fever antibodies in their sera.

All captured animals were sent to the field laboratory and bled shortly after arrival. Their sera were sent to the central laboratory for testing for yellow fever antibodies. The marmosets were kept under observation for a minimum period of eight days, and were then preserved for use as experimental animals, provided that they were non-immune. Any which died were carefully autopsied. Portions of liver, lung, spleen and kidney were sent to our central laboratory for pathological diagnosis. If, at time of autopsy, the gross appearance suggested infection with yellow fever virus, a suspension of the liver and, occasionally, blood serum and brain material were inoculated intracerebrally into mice. In certain instances non-immune marmosets were inoculated subcutaneously with similar material.

Almada strain 1. Marmoset A was captured in a small patch of forest situated near the railway station at Lava Pés, on property belonging to the Fazenda Almada. The animal was captured on June 7, 1944. The following day it was received at our field laboratory, where it was noted as being "in a weak condition." A blood sample was withdrawn from the heart on June 8, and 4 hours afterward the marmoset died. On autopsy the gross pathology was recorded as "suspicious for yellow fever." This diagnosis was confirmed by histological examination. A 10 per cent suspension of liver tissue was prepared, and the centrifuged supernatant was injected intracerebrally into mice. Serum obtained on June 8 contained yellow fever antibodies and also an antigen capable of fixing complement in the presence of yellow fever antiserum.

One of the six mice inoculated with the liver suspension became ill on the ninth day after injection. Its brain was passed to a second group of five mice, three of which became ill on the fifteenth day. The brains of these sick mice were removed, emulsified in saline and centrifuged. A portion of the supernatant was used for a specificity test, which confirmed the identity of the pathologic agent as yellow fever virus; the remainder was desiccated and sent to our central laboratory for preservation.

Among the remaining five mice of the original group inoculated with liver suspension, two became ill on the twelfth day, one on the eighteenth day and one showed no sign of illness during a 30-day observation period.

[1]The work on which these observations are based was done under the auspices of the Serviço de Estudos e Pesquisas sôbre a Febre Amarela (Yellow Fever Research Service) which is maintained jointly by the Ministry of Education and Health of Brazil and the International Health Division of The Rockefeller Foundation.

Bomfim strain. On August 7, 1944, Marmoset B was captured in a cocoa plantation at Fazenda Bomfim, about 3 kilometers distant from the place where infected marmoset A had been captured. It was received at the laboratory on August 10, in a weak condition, and died after being bled on the same day. Gross autopsy findings suggested yellow fever infection. One group of mice and one marmoset were inoculated with serum and a second group of mice and a second marmoset were inoculated with a 20 per cent liver emulsion. This serum contained yellow fever antibodies and complement fixing antigen. Histological examination of liver sections revealed slight fatty degeneration and scattered necrotic parenchymal cells.

Of the five mice inoculated with serum, two died and three were sick on the seventeenth day after injection. Passages from the brains of the sick mice confirmed the identity of the virus as that of yellow fever. Of the six mice injected with liver suspension, one died and two were ill on the seventeenth day; the remaining three showed no sign of illness.

The marmoset injected with serum circulated virus and became immune. It died, of extraneous causes, 27 days after injection. The marmoset receiving the liver emulsion survived and became immune. No virus was demonstrable in its serum.

Almada strain 2. Marmoset C was captured on August 10, in a cocoa plantation at Fazenda Almada. This plantation is continuous with the one belonging to the Fazenda Bomfim, and the capture of this marmoset was made only 500 meters from the point where Marmoset B had been captured. The marmoset was received at the laboratory in a weak condition and died while being bled. The gross pathology was suggestive of yellow fever infection. The serum gave a partial protection (3/6) and contained complement fixing antigen. The pathological diagnosis was negative for yellow fever. A centrifuged supernatant from a 20 per cent emulsion of liver tissue was injected into mice. All five mice in the group became ill on the twelfth day, and tests with emulsions of their brains confirmed the diagnosis of yellow fever virus infection.

Almada strain 3. Marmoset D was captured on August 13 in the same trap as was Marmoset C. It was received at the laboratory on August 15, in poor physical condition, and died following bleeding. Again, the gross pathology was suggestive of yellow fever infection, and this was confirmed by histopathological diagnosis.

The supernatant from a 20 per cent liver suspension was injected into five mice. One died and four became ill on the eleventh day after injection. From the brains of the sick mice this strain of virus was established and identified.

DISCUSSION

It is of interest to note that this small epizootic was sharply delimited, both in time and in location. All of the infected marmosets were captured between June 7 and August 13, although our investigations have extended over a period of more than one year. So far, 1,437 marmosets have been obtained and no additional strains of virus have been encountered.

With regard to geographical distribution, all of the four infected marmosets were captured at points not more than 3 kilometers apart, in a locality at the extreme northern boundary of our study area. The latter is in the form of a rectangle with sides about 40 by 30 kilometers.

The strains of virus obtained, as described above, exhibit some peculiarities in pathogenicity. The investigation of these viruses is under way in our central laboratory and will form the basis of a future communication.

SUMMARY

Yellow fever virus has been isolated on four occasions from wild marmosets, *Callithrix penicillata*, in a restricted locality situated in a region where jungle yellow fever is endemic.

THE TEACHING OF TROPICAL MEDICINE IN THE UNITED STATES[1]

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This subject demands consideration from several points of view:

I. The education of the present-day practising physician.

II. The education of the present generation of medical students.

III. The special training of the medical personnel of the fighting forces.

IV. The education of future generations of medical students.

V. The training of medical personnel for civilian service in tropical countries.

Although there is some overlapping of interests, both the conditions that necessitate action and the action that should be taken under each of these headings are different. It will, therefore, be best to consider each case separately.

I. EDUCATION OF THE PRESENT-DAY PRACTISING PHYSICIAN

In the immediate future and for several years after the war, practising physicians in this country will certainly encounter many instances of tropical disease among returned personnel of the fighting forces and among civilians whom the war has taken into tropical countries. There is also a considerable danger that new infections and new strains of established infections may be introduced into this country by returning service and civilian personnel. Finally, the increase in volume and speed of traffic between this and tropical countries that is certain to follow the war will maintain this closer contact with tropical diseases and prolong indefinitely the danger from the introduction of new infections.

The education of these practitioners is made necessary not only because tropical medicine is a rapidly advancing subject about which those doctors who have settled down to practice in this country will naturally not have kept themselves up-to-date, but because many of them probably went through their medical training without hearing even the names of some of the tropical diseases that have become important today. This defect in medical education is the result of a parochial attitude towards the science of medicine that has hitherto prevailed; it is by no means peculiar to the United States; it is understandable in view of the rarity of tropical experience among those responsible for the medical schools' curricula but it is none-the-less inexcusable.

It will of course be out of the question to consider any extensive post-graduate education for the present generation of practitioners; their immediate needs can probably be best met by means of reviews and symposia in medical journals, lectures arranged through local medical societies, and short post-graduate courses. The aim of these post-graduate courses should be not to turn the practitioner into an expert in any particular tropical disease or group of diseases, but to give him an over-all picture of the subject.

(a) to make him aware of the existence of the more important tropical diseases and of the possibilities of their turning up in his practice,

(b) to remind him of the methods of diagnosis that are available, but without necessarily instructing him in these methods, and

(c) to outline the main methods by which such diseases are treated and controlled.

Much has already been done in this matter and there is considerable awareness of the need for extending this education, so that I shall pass on to the next heading.

II. THE EDUCATION OF THE PRESENT GENERATION OF MEDICAL STUDENTS

This is necessary for all the reasons given above, and also because the majority of these men will be going into the armed forces and will be liable to see service in the tropics.

It is obvious that at short notice it is not possible to make the fundamental alterations in the curriculum that would remedy its defect in the most satisfactory way, and the plans for meeting the emergency that were worked out by the Committee on the Teaching of Tropical Medicine in Under-

[1] Read at the Fortieth Annual Meeting of the American Society of Tropical Medicine, St. Louis, Mo., November 13–16, 1944.

graduate Medical Schools of the U. S. were, in my opinion, the best that could have been formulated in the circumstances. They have been extremely successful in practice, but I believe that the members of this committee will agree that their plans were only a temporary expedient.

Selected teachers from medical schools have received a two-months course of instruction in tropical medicine and parasitology at the Army Medical School at Washington and at Tulane University, and a shorter course of field instruction in a tropical country, namely, Central America. Despite shortage of staff and the difficulty of sparing their teachers, most of the schools in the country have cooperated in this scheme and sent teachers for instruction. These teachers have organized and have themselves conducted lectures and classes in tropical medicine and parasitology. The special classes have usually been dovetailed in with existing laboratory and clinical classes and have seldom been presented courses of instruction. In addition, further to stimulate interest in the subject, visiting lecturers with actual experience in the tropics have been invited to deliver special lectures on tropical diseases at the medical schools throughout the country.

The effect of this broadening of the outlook of medicine, which will be aided by the inevitable increase in instances of tropical diseases in the hospitals associated with these teaching institutions, will be felt for some years, but unless a new spirit is infused into medical thought and education as a whole, and permanent changes are made in the curriculum, there will be a relapse into the pre-war state of indifference towards tropical diseases, until such time as we are jerked out of it by some incident similar to the one at Pearl Harbor on December 7th, 1941.

III. THE SPECIAL TRAINING OF THE MEDICAL PERSONNEL OF THE FIGHTING FORCES

Training is required for selected members of the medical personnel of the armed forces for special work in tropical fields in which they are likely to be operating; for example, in malariology in all its aspects, general protozoology, field and laboratory entomology, helminthology, mycology, virology, and field sanitation in the tropics. This need not be discussed here as it is being well undertaken by the several services.

IV. THE EDUCATION OF FUTURE GENERATIONS OF MEDICAL STUDENTS

It has been suggested above that the present limited outlook in medical education is inexcusable, that the patched-up medical course which has served excellently to meet immediate requirements cannot be accepted as a final solution to this problem, and that a thorough revision of the curriculum is necessary.

This criticism of the present attitude of medical faculties towards tropical infections is by no means directed at the faculties in this country; the attitude is much the same in Great Britain and Canada, and even in India, where it is not only inexcusable, but incomprehensible, in view of the fact that the country is largely a tropical one. It is perhaps unfair to blame medical faculties. The present attitude of the medical profession as a whole towards medical science is far too frequently governed by immediate practical considerations, to the grave detriment of the science, as a science.

The basic objective of medical science is the study of Man (genus *Homo*) and his reactions to environment, parasitization, and degenerative processes. These reactions when they reach a certain level of intensity are known as diseases. We should not be content to study only the diseases that are likely to occur in our own town and in the neighboring towns, in our own state and the surrounding ones, or even our own country, nor those diseases that occurred yesterday and therefore likely to occur today and tomorrow, but our interest should embrace diseases of all countries and all times, past, present and future. The parasites and the environmental factors may be different from place to place and from time to time, but the human organism is fundamentally the same throughout the world, and what is learnt from the reactions of Man to one pathogen will in innumerable ways be of value in studying his reactions to another.

There are many eminent internists and pathologists, who, while priding themselves on their knowledge of rare syndromes of which there may have been only a few dozen cases described in the world's medical literature, will shamelessly admit that they know nothing about such tropical diseases as cholera, sleeping sickness, kala-azar and schistosomiasis which with great regularity take their annual toll of tens of thousands of

victims. They will admit that their knowledge of malaria, a disease that affects a quarter of the inhabitants of the globe and causes more morbidity than any half dozen other diseases put together, is limited to a little rudimentary information regarding the periodicity of the fever and the specificity of quinine.

There is of course always a place for the specialist, the man who after receiving a thorough basic training makes a special study of certain limited aspects of disease, but how can the physician or the pathologist, who is teaching the new generation the principles of the body's reactions to parasitization afford to ignore those reactions that are produced by certain bacteria and viruses and by whole groups of parasites such as the protozoa and the helminths, on geographical considerations alone? From a purely scientific point of view, though admittedly not from a practical one, it would be as logical for him to say "This subject is too big to tackle as a whole, so we will stop at the letter R and omit all reference to diseases beginning with the letters S to Z".

No study can be considered complete without bringing forward all available evidence, and the evidence on the results of parasitization and specific malnutrition of the human organism is to be found abundantly in almost every tropical country; there are quantities of scientific treasure on or near the surface that are only waiting to be recognized and collected.

You may perhaps ask, why then has it not been collected many years ago by the medical men who have spent the best parts of their lives in the tropics? It is perfectly true that many opportunities have been neglected, but workers in the tropics have not been idle during the last fifty years. Volumes could be written on the debt of 'cosmopolitan' to 'tropical' medicine; three outstanding examples will suffice: (i) medical entomology, protozoology and helminthology, if they did not originate, at least graduated in the tropics, but are now being practised with almost equal effectiveness in temperate zones, (ii) all the early experimental work and the vast majority of the early triumphs in chemotherapy were in the treatment of tropical infections, and (iii) the study of the vitamins commenced in the tropics.

The parasitologist has had his day; he has garnered considerable treasure and, although he has much work still to do, especially in mycology and virology, just as great opportunities for important original work today await the well-trained physiologist, biochemist, pathologist, and internist. Few such men have been attracted to the tropics, but those who have gone there, even on short tours, have been amply rewarded.

The place of tropical diseases in the curriculum. The prospective medical student might well be introduced to systematic parasitology in his premedical years; special attention could be paid to the groups that parasitize man. There should be no difficulty in introducing a broader programme of instruction in medical parasitology into the earlier years of the medical course; it has never been logical to choose bacteria out of all the parasites for special emphasis, and the bacteriology classes could be extended to include the study of other fungi, viruses, rickettsiae, protozoa and helminths, with considerable benefit to the interest of the course.

As our knowledge on the immunology and pathology of protozoal and helminthic infections is less standardized, these subjects will present greater difficulties, but a beginning could be made and the obvious lacunae in our knowledge might be emphasized in order to stimulate research.

The clinical aspects of tropical diseases, both parasitic and dietetic, should certainly take their place in the general medical classes, and, while it would perhaps be permissible for a time to assign some of the teaching on tropical diseases to a member of the staff who had had tropical experience or who had made a special study of these diseases, any real segregation of the subject should be avoided, and other members of the staff should endeavour at least to conceal their ignorance.

Teachers of internal medicine will usually raise the objection that clinical material is not available for teaching of tropical medicine. However, it must be remembered that there are cosmopolitan diseases about which the student has to be taught without the aid of clinical material, and admittedly many tropical diseases will have to be added to this list. Much can be done by means of latern slides, particularly coloured slides, and the moving picture to make these diseases, and the conditions under which they occur, more real to the medical student. The provision of such material by the Army Medical Museum has been of the greatest help to medical schools, but there should be some civilian organization to continue and extend this service.

Finally, the eminently preventable nature of

most tropical diseases should make them welcome to the teacher of preventive medicine to demonstrate what can be and has been done in the way of controlling disease.

In the course of a generation of medical students, this should break down the purely artificial barrier that exists today between tropical and temperate medicine; it will certainly greatly benefit the study of so-called tropical diseases, but almost as certainly it will have very valuable repercussions on the study of cosmopolitan diseases.

V. THE TRAINING OF MEDICAL PERSONNEL FOR CIVILIAN SERVICE IN THE TROPICS

The need for a school of tropical medicine. The need for such a school in this country has been felt for some time, but will be far greater in the near future. Beyond the officers of the Rockefeller Foundation, a fairly large number of medical missionaries, and the medical officers of a few industrial and commercial concerns, such as the United Fruit Company, the Standard Oil Company and the Pan-American Airways, very few medical men have sought civilian employment in foreign countries.

The war will undoubtedly lead to more foreign enterprise on the part of the industrial and commercial interests in the United States, and, as the natural resources of this country begin to approach exhaustion, this tendency will increase. This change will be aided very considerably by the vastly improved travel facilities that will follow the war. Americans, who have hitherto hesitated to cut themselves off from their homes and families for long periods, will take foreign appointments much more readily when they know that they can fly home within 60 hours from any country in the world, and, in fact not an unimportant part of foreign medical work will actually be in connection with the numerous airdromes with which the world will be dotted.

The importance of properly trained personnel. It will be very necessary to take active measures to impress upon those responsible for new industrial and commercial enterprises the great importance, from a financial as well as a humanitarian point of view, of guarding the health of their employees, both the natives of the country and American overseers and their families, and to emphasize the fact that this can only be done satisfactorily by employing medical men especially trained for this work.

Mistakes of the past. Although there have been for many years, two good schools of tropical medicine in Great Britain, until recently most British industrial concerns recruited any physically and temperimentally suited medical man who was willing to go out to the tropics, without demanding that he should have any special training or tropical experience. These men had to learn their tropical medicine the hard way, from their similarly untrained senior colleagues, from unguided practical experience, and from books, which often appeared to them to be very misleading.

In such circumstances, the majority, either finding a great disparity between precept and practice and failing to see the significance and opportunities of practice in the tropics, became discouraged and resigned their appointments, or they developed an unhealthy scepticism towards medical science, and, taking all possible short cuts in the treatment of their patients, resigned themselves to a life of comparative ease. Others have of course been able to carve out a course for themselves despite the disadvantages, and have made notable contributions to medical science.

Further, the medical officer frequently found himself isolated from medical colleagues and at a great disadvantage with the manager of the industrial concern, who during his long service in the tropics had often acquired a surprising, if superficial, knowledge of tropical medical practice. Under these conditions, a medical officer had great difficulty in recovering his moral ascendency over his lay colleague, so that later he found it impossible to impress upon him the necessity for adopting any new sanitary measures which he himself might consider were indicated. Local managers usually tended to oppose change, especially that involving expenditure, and they therefore generally favoured an easy-going attitude in the medical officer.

There has however been a considerable change in recent years and in many instances the headquarters directorates of tropical industrial and commercial concerns in Great Britain have appreciated the advantage of employing trained men and of adopting the suggestions of these men, not only for the immediate improvement in health of their employees, but for long-term health policies. The intelligent medical officer will usually appreciate the fact that wherever he goes he has still much to learn regarding special local conditions, but a thorough basic training in tropical medicine will give him the self-confidence

that will enable him to take advantage of the valuable local knowledge of his non-medical colleagues without losing his self-respect.

Other prospective candidates. Missionary organizations have usually realized the necessity for giving their medical officers special training and have sent their personnel to take appropriate courses wherever these were obtainable[2]. It is certain that these organizations would welcome the opportunity of sending their medical men and women to take a comprehensive course of tropical medicine in the United States.

I do not propose to give a long list of the classes of individuals who will be interested in a course of tropical medicine, but perhaps foreign visitors from tropical countries, especially Central and South American countries, should be mentioned as likely to seek admission to such courses. For both political and commercial reasons these will undoubtedly be welcome.

The syllabus

There has been a tendency in the past to consider that the only special training necessary to prepare a man for practice in the tropics is a course of parasitology with perhaps a little field malariology thrown in. This is a narrow and out-dated point of view, but it is a firmly established one that will die hard. The place of parasitology in tropical medicine is an important one, but it must not be allowed to dominate the course, and as the general standard of undergraduate teaching in parasitology improves it may be possible to reduce this part of the course.

The syllabus should include as a minimum the the following subjects:

(i) *Climatology and the geography of the tropics* including some geology, simple data regarding rainfall of representative tropical areas, and the effect of the telluric and climatic factors on 'staples' and other food supplies, and in a general way on the distribution of disease.

(ii) *Ethnological studies* of the peoples in different tropical countries, with some references to their specific immunity and susceptibility to certain infections.

(iii) *Economics* of certain selected tropical countries, with special reference to the current standard of living of the population and the possibilities of improving this by non-medical, e.g. irrigation and agricultural development, as well as by medical measures, and to the extent to which the country can afford to meet the initial and recurring costs of sanitary improvement. This might also be applied to comparatively rich industrial undertakings, e.g. mines and plantations within these same countries, and contrasts made.

(iv) *Medical organization in tropical countries.* Orientation on the types of organization for medical relief and prevention existant in representative tropical countries.

Public health in the tropics

(v) *Vital statistics, and mortality and morbidity data.* The methods of collecting these and their relative value in tropical countries.

(vi) *Medical records.* Methods of case recording, making sickness returns, and the collection of other data under tropical conditions.

(vii) *Simple statistical methods* and ways of appraising the collected data.

(viii) *Education and propaganda.* The possibilities of these as instruments of the public health worker in populations of different grades of education and educability.

(ix) *Housing, hospital construction, sanitation, and water supplies and malarial engineering,* in selected tropical countries.

(x) *Measures for mitigating the effects of heat, and the maintenance of health in the tropics.*

(xi) *Epidemiology,* generally and with special reference to certain selected tropical diseases.

(xii) *Special measures of disease control.* General discussion on specific treatment, animal reservoir destruction, vector destruction and vaccination, as measures of control.

(xiii) *Physiology* of hot climates, including the symptomatology and treatment of all conditions due *directly* to the effects of a hot climate, e.g. heat stroke.

(xiv) *Nutrition.* Nutritional requirements of different population groups in the tropics. Tropical dietaries in representative selected areas. Common deficiency states and diseases of dietary origin (or apparently of dietary origin), e.g. sprue, beri-beri, pellagra, epidemic dropsy, that are common in the tropics.

(xv) *Zoology.* Classifications and general description of morphology and habits of the commoner animal reservoirs of infection of tropical diseases.

(xvi) *Entomology.* Classifications and general description of the morphology and ecology of vectors of pathogenic organisms, of filth flies,

[2] Several American medical missionaries have taken the course at the Calcutta School of Tropical Medicine.

and of insect pests, the identification of the important mosquito genera, and the use of "keys" for identifying larvae and adults of anopheline species.

(xvii) *Parasitology, including the study of helminths, protozoa, rickettsiae, spirochaetes, bacteria and other fungi, and viruses.* This should cover classification, morphology and life cycles of important pathogenic genera and, in some instances, selected species, the host-parasite relationships and the immunological reactions of man with reference to the various groups of parasites, methods of identification of the commoner pathogenic species and diagnostic procedures.

This course should consist of lectures, demonstrations, and practical exercises. Special emphasis should naturally be given to the commoner species that are usually classed as tropical, e.g. to hookworms amongst the helminths, to the malaria parasites and the pathogenic amoebae amongst the protozoa, and to the dysentery organisms amongst the bacteria. The rarer helminths and fungi should be treated in groups, and as this is to be a post-graduate course, it should be possible to assume that the candidates know at least the elements of bacteriology.

(xviii) *Herpetology.* Including shorter references to other venomous animals.

(xix) *Pathology.* This should be both human and comparative. The wild reservoirs of infection and laboratory animals will form the main subjects for the latter. (Teachers will at first be hampered by shortage of material and data, as this aspect of tropical medicine has been grossly neglected).

(xx) *Haematology, clinical laboratory tests and biochemistry.* This subject will have to be strictly limited, but there are certain special investigations that are more commonly used in the tropics.

(xxi) *Pharmacology and therapeutics.* Pharmacological research on drugs of established value, and on new drugs in the experimental stages will be an important function of the school, and some instruction in methods could appropriately and easily be given. The teaching on therapeutics should be general and apply mainly to groups of specifics; it should include discussions on the mode of action of these drugs, mass treatment and drug prophylaxis.

(xxii) *Tropical medicine. This should consist of correlating lectures on individual diseases, in which the disease is reviewed as a whole.* These should include history, aetiology, epidemiology and prevention, pathology, symptomatology, diagnosis, treatment, and prognosis, but with special emphasis on the clinical aspects. It will be essential that the lecturer is fully conversant with the teaching of other departments, in order that he may correlate this but avoid unnecessary repetition.

(xxiii) *Clinical teaching,* whenever material is available. In the post-graduate teaching of tropical medicine, clinical instruction on actual cases in the wards is very important, but, except with reference to certain diseases such as leprosy, its value is to a large extent psychological. It should not be necessary to teach the post-graduate student how to take case notes and examine a patient. The patient in hospital in a temperate country often presents a very different clinical picture from a patient with the same disease in his home in the tropics, so that by presenting such a case a very poor visual impression of a disease is often created. Nevertheless, to have seen a patient with a disease, however atypical it may have been, gives the student something concrete that will stimulate greatly his interest in that disease and add reality to his subsequent reading on the subject. There is however another side to this and there are times when the patient chosen as the subject is so far removed from the real thing that the situation becomes both ludicrous and misleading; such an example was a clinic given by a London physician on hookworm disease by the bedside of an Indian lascar (sailor), convalescent from malaria, who happened to pass a few hookworm eggs. On balance, a "dry" clinic by a physician who has had experience of the disease in the tropics is infinitely preferable to a "wet" clinic given by a stay-at-home physician who has seen only a few similar cases in his own hospital.

(xxiv) *Tropical skin diseases.*

(xxv) *Surgery in the tropics* and special surgical procedures for tropical conditions.

(xxvi) *Obstetrics* and special problems presented by women in the tropics.

(xxvii) *Tuberculosis in the tropics.*

(xxviii) *Venereal diseases in the tropics.*

(xxix) *Psychiatry in the tropics.* The study of so-called tropical neurasthenia, and discussions on the incidence of various psychiatric conditions in natives of tropical countries and the facilities for treatment and control of patients.

(xxx) *Special lectures on the blanks and hiatuses in our knowledge of tropical diseases.*

(xxxi) *Nursing in tropical countries* and the training of native personnel.

(xxxii) *Medical administration in the tropics*, with special reference to the obtaining and care of stores and equipment.

It will be obvious that the amount of time to be devoted to each of these subjects will be different; it should vary from, say, 20 to 30 per cent of the total time allotted for the whole course to parasitology and about 20 per cent to tropical medicine and clinical instruction, to, say, 1 to 2 per cent to such subjects as climatology.

The course should be made comprehensive in so far as tropical diseases and disease-producing parasites are concerned, but there are several subjects, principally those under the heading 'public health', in which it will be necessary to select a few representative tropical areas for discussion, partly on account of the time factor, and partly because data for many areas will not be available, at least at first. Although much of the data presented may be of little practical value to a doctor going to quite a different tropical environment, it will make him relaize the value of such data and encourage him to collect it in his own locality. It should be the aim of teachers to complete their data for as many areas as possible, as time goes on, perhaps with the help of their students, with all of whom they should endeavour to maintain touch, so that in time the school will be recognized as a source of reference on such matters.

A course of 8 or 9 months with about 1,000 hours of instruction would be appropriate.

Staff

Such a wide range of subjects could only be taught satisfactorily in a large university. It will almost certainly be found advisable to seek the assistance of members of the university outside the medical faculty for some of the subjects.

It will be necessary to have certain independent departments of the school of tropical medicine, some of whose members should be available to take part in the undergraduate teaching in the medical school also, but for the rest the tendency should be to attach special assistants to the existing department of the medical school rather than to start innumerable separate departments in the school of tropical medicine. This would have the double effect of economy and the stimulation in these departments of interest in tropical medicine. These men(or women) whenever they are at headquarters would take up the ordinary work of the department as well as their special subjects, but they should not be tied down and should be free to do field work in the tropics when opportunity arises.

The full-time staff should include:

A professor of tropical medicine (with tropical experience) and at least two assistant professors; any of these could take part in the teaching of tropical subjects in the undergraduate course of the medical school.

A professor of public health (with tropical experience) and one or two assistants who could take care of all the subjects under the heading of public health.

A professor of parasitology (preferably an M.D. with tropical experience) and two or three assistants (who could be Ph.D's), preferably specialists in different subjects, but with sufficient knowledge of the other subjects to be able to take over the teaching of these if necessary. These staff members should help in the teaching of the undergraduate students.

A medical entomologist (with tropical experience) and an assistant. Either one should be able to undertake the teaching with the aid of a technician, so that the other could be free to carry out field work. This department might also undertake instruction in Zoology.

An epidemiologist and medical statistician, who should work with both the professor of public health and the entomologist.

A biochemist who could work partly, but not solely, on nutritional problems.

There should be one full-time member of the staff with experience in an industrial concern in the tropics, and another should be a woman doctor (M.D.) with tropical experience, as there are problems, not only in obstetrics, but in nutrition, education and propaganda, housing, psychiatry and general sociology on which a woman's point of view is necessary.

The instruction in pathology should be undertaken by the pathological department of the medical school, but they should have two assistants especially for this work, one for teaching and the other for field work, interchangeably.

The pharmacology department of the school should similarly be strengthened by one or two special assistants who would also undertake research work.

Instruction in nutrition, tuberculosis, skin diseases, venereal diseases, surgery, obstetrics, and psychiatry should be undertaken by the appropriate departments of the medical school, but in

each case an assistant should be attached for special study; these lectures could be given within a short period during the course, leaving the assistant free at other times for tropical field work.

Physiology should be taught by that department of the medical school, but special studies of the physiology of warm climates should be made.

This leaves several other minor subjects to be taken up by other members of the faculty competent to teach these subjects.

In all instances, the members of the staff should have spare time to devote to research work at headquarters, and should not be tied for the whole year, in order that they might periodically visit and carry out investigations in tropical countries. Perhaps also it would be possible to arrange exchanges of personnel with institutions in tropical coutries, e.g. China, Java, India or tropical America, but it would not be advisable to become committed to support a permanent institution in any one country, as this would tend to limit the interests of the staff to the diseases in this one country and would thus defeat one of the main objects of the school. The school might also maintain a close liaison with some organization that is undertaking antimalarial work in this country, for field-training of their students.

Training of other medical personnel. While the principle work of the school should be the training of medical men and women, an important side-line should be the training of malaria field-workers, sanitary engineers, technicians, and nurses for service in the tropics. Candidates for these courses should be men and women of a high standard of education and they should be taught with the idea that their main work will be the teaching of native personnel.

The number and location of the schools in the United States. While I feel convinced that the medical school in any university that establishes a school of tropical medicine within its walls will be immeasurably benefitted, I consider that in the first instance only two or at most three such schools should be opened in the United States.

The actual location of the school (or schools) should be governed by the size and importance of the teaching institution with which it would be associated, and the availability of clinical material, in that order.

The diversity of the subjects to be taught and the help that will be required from other members of the staff of the university make it imperative that it should be a large one, and the special studies that will have to be undertaken make it necessary that the members of the staff should have plenty of spare time and liberal funds for research work.

The extreme importance of clinical teaching cannot be overemphasized, and for that reason the school should be located near a large port, but the fact remains that, even if one could organize a mobile school of tropical medicine and travel around the world, there would still be many diseases about which the student would have to learn solely from lectures and demonstrations. In any stationary school even in the tropics, one can never hope to show more than, say, a third of the diseases on which instruction will have to be given. Important though it is, availability of clinical material should not therefore be allowed to outweigh all other considerations.

I would like to make one final suggestion—that when the first tropical school is established in the United States, the motto of the school should be "Unus Mundus et unus Homo"—One World and one Man.

CHEMOTHERAPY OF EXPERIMENTAL HISTOPLASMOSIS IN WHITE MICE[1]

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INTRODUCTION

The purpose of this paper is to describe observations made during a study of experimental infection and treatment of mice with histoplasmosis.

Histoplasmosis, a fatal fungous disease, is being recognized in an ever-increasing number of cases. The disease is characterized clinically by a low grade, irregular pyrexia, splenomegaly, hepatomegaly and lymphadenopathy. Anemia and leukopenia are observed in most cases. Ulcerated granulomatous lesions of skin, oral mucous membranes, pharynx and gastrointestinal tract occur. These organs and tissues are seldom affected uniformly. The disease may be localized or generalized. Signs and symptoms are related to the organ system involved. The disease may last from a few weeks to many years. Regardless of the time interval, the fungus develops until death intervenes. Table 1 demonstrates the wide variety of clinical and pathologic lesions observed.

The etiologic agent is a cultivable fungus, *Histoplasma capsulatum*. The organism produces yeast-like bodies during the parasitic phase of its life cycle. It exists in tissues as small, oval yeast-like bodies, measuring 3–3.5 micra in diameter. Each cell is surrounded by a clear, nonstaining membrane, or "halo". In preparations stained with hematoxylin and eosin the nucleus usually appears as a peripheral, pink stained crescent shaped mass. The yeast-like form will persist on sealed blood agar slants at 37°C. The mycelial form of the fungus develops from yeast-like cells when they are transferred to a simple sugar containing agar medium and kept at room temperature. This form develops by elongation of yeast cells, the hyphae soon becoming septate.

Clinically, many forms of treatment have been tried, unsuccessfully. These include fuadin, neostam, sodium iodide, neoarsphenamine, bismuth, pentnucleotide and liver extract, sulfanilamide, atabrine, prontylin, potassium arsenite, quinine, vitamins, sulfathiazole, sulfapyridine, emetine, sulfarsenal, and others (1). The disease has been produced experimentally and studied in animals, especially the white mouse (2, 3). No attempt at chemotherapy in animals has been reported.

Reid et al. (4) had the opportunity to study, at the Medical College of Virginia, a case of histoplasmosis which terminated in the death of the patient. The organisms used in this work were obtained from this source.

One hundred and forty-five mice were injected intravenously with the fungus, *Histoplasma capsulatum*, and treatment was started within twenty-four hours after this injection.

Eight drugs were used in this study; sodium iodide, neostam, fuadin, sulfanilamide, proflavin, thymol, B-9,[3] and sodium propionate. The results indicated that none of these drugs are useful in the treatment of histoplasmosis in mice.

REVIEW OF LITERATURE

The disease in man

The literature on histoplasmosis in the human has been adequately reviewed by Moore and Jorstad (1) and Meleney (5). Table 1 summarizes the available reports.

The disease in animals

The only case of a natural infection in animals was reported by DeMonbreun (6). This occurred in a three year old, male Boston Bull terrier. A biopsy of the liver revealed yeast-like parasites in Kupffer cells and macrophages in lesions involving the portal areas. A tentative diagnosis of Histoplasmosis was made.

The autopsy diagnosis was: Histoplasmosis, involving liver, spleen, mesenteric lymphnodes, peritoneum, gastro-intestinal tract, lungs, heart

[1] The data presented are taken from a thesis submitted to the Faculty of the Graduate School of the Medical College of Virginia in partial fulfillment of the requirement for the degree of Master of Science.

[2] Fellow in the division of Graduate Study, Medical College of Virginia, School of Dentistry.

[3] B-9 is an organic iodide prepared by Dr. Berger, Dept. of Chemistry, University of Virginia.

TABLE 1

Summary of case reports available in the literature on histoplasmosis

AUTHOR	VITAL STATISTICS				ORGANS INVOLVED										
	Location	Age	Sex	Race	Skin	Naso-oral cavity	Lung	Liver	Spleen	Adrenal	Node	G.I. tract	Marrow	Heart	Other
		years													
Darling (8)	Canal Zone	27	M	Negro			x	x	x		x		x		
Darling (9)	Canal Zone	29	M	Negro			x	x	x		x		x		
Darling (9)	Canal Zone	55	M	Chinese			x	x	x		x		x		
Watson (19)*	Minnesota	52	F	White			x		x		x				
Phelps (20)*	Honduras	24	M	Honduras			x								
Wade (21)	Philippines	40	M	Philipp.	x	x	x	x	x		x				
Crumrine (22)*	California	42	M	Negro			x	x	x		x	x			
Müller†	Java	7	M	Javanese							x	x			
Dodd (12)*	Tennessee	0.5	M	White	x		x	x	x		x	x	x	x	Ear
Hansmann (23)*	Iowa	43	M	White	x	x	x	x		x	x				Ear
Agress (24)*	Missouri	0.18	M	White		x	x	x	x		x	x		x	
Amolsch (25)*	Michigan	0.68	F	White					x		x	x	Smear, no autopsy		
Shaffer (26)*	Virginia	1	F	White	x		x	x	x		x	x			
Negroni (27)	Argentina	40	M	White		x	x	x							
Clemens (28)*	Kentucky	33	F	Negro		x	x	x	x	x		x	x		Pancreas, kidney
Gunter (29)*	Alabama	54	F	White			x	x	x						
Humphrey (14)	Michigan	17	M	White		x	x	x	x		x				
Humphrey†	Michigan	46	M	White	"Generalized"										
Williams (30)*	Tennessee	56	M	White		x									
Parsons†	Michigan	67	M	White											
Willer†	Ohio	12	M	White	"Generalized"										
Forry*†	Indiana	10	M	White	"Generalized"										
Martin*†	California	43	F	White			x	x		x					
Currie†	Indiana	62	M	White						x					
Vilella*†	Brazil	3	F	Mulatto	"Generalized"										
Meleney (33)	Tennessee	50	M	White			x								
Meleney (33)	Tennessee	69	M	White			x								
Blache*†	Missouri	33	M	Negro			x								
Reid (4)*	Virginia	38	M	Negro			x	x	x		x	x	x		Pancreas, kidney
Mantell*†	Florida	42	M	White		x	x	x	x		x	x	x		Kidney
Wright (36)*	Maryland	59	M	White			x	x	x		x				
Almeida (37)*	Brazil				x		x	x	x		x				
Brown (38)*	Texas	48	M	White		x	x	x							
Anderson (39)*	Tennessee	0.68	F	White	x		x	x	x		x	x	x		Ear, kidney
Balina (40)*	Argentina	33	M	White	x	x	x	x	x		x				
Simson (41)*	Cape Province, Africa	55	M	White		x		§			§				
Dean (42)	Missouri	41	M	White	x	x	x								
Van Pernis (43)	Illinois	65	M	White		x	x	x	x	x	x				
Moore (1)*	Missouri	67	M	White		x	(no autopsy)								
Sherwin‡	Missouri	71	M	White		x	(no autopsy)								
Parsons‡	Michigan	25	F	White	x	x	(no autopsy)								
Parsons‡	Michigan	49	M	White		x									
Palmer (18)*	Michigan	45	M	White		x	x		x	x		x			Penis, kidney
Broders (47)*	Minnesota	47	M	White				x	x					x	Kidney
Rhodes (48)	Ohio	0.25	F	White	x		x	x	x						Pancreas, kidney
German (49)*	Ohio	0.43	M	White			x	x	x		x	x		x	Thymus, kidney
Burden*‡	New York	68	M	White		x				x	x				
Dominguez*‡	Ohio	53	M	White		x	x	x	x					x	Parotid, kidney
Key (7)*	Missouri	47	M	White			x								Knee jt.
Derry (44)	England	30	M	White							x	x			Tonsil
Henderson (45)	Michigan	70	M	White			x	x	x	x	x				Kidney
Hild (46)	Texas	0.43	F	White	x		x	x	x	x		x	x		Kidney
Scott (35)	Kentucky	1.25	M	Negro			x	x	x		x	x	x		
Thomas (34)	N. Carolina	44	M	White			x	x	x	x	x				Kidney
Boltjes (32)	Kansas	42	M	White		x				x					

* Senior author.
† By Meleney (5).
‡ By Moore and Jorstad (1).
§ Palpable.

and meninges. The organism, *Histoplasma capsulatum*, was recovered from the blood drawn from the dog the day previous to his death, and from the ascitic fluid, liver and spleen tissue taken at autopsy. The media from which this isolation was made was infusion broth (pH 7.2), potato-dextrose agar (pH 6.5), 10% rabbit blood agar, and N.N.N. medium.

DeMonbreun transmitted the disease to dogs and puppies by feeding them cultures of the fungus. He was also able to infect puppies by injecting a saline suspension of organisms intraperitoneally. In all cases, lesions similar to those described in the "natural" infection were found.

Tager and Liebow (3) made a study of induced infection in mice with *Histoplasma capsulatum*. They were able to produce the infection by intraperitoneal injection of the mycelial form of the fungus. Except for failure to produce granulomatous lesions, the pathologic changes were analogous to those found in man. There was hyperplasia of the reticulo-endothelial system, the cells containing large numbers of the yeast form of the organism. The spleen and liver were enlarged, but there was no fluid in the peritoneal or pleural cavities.

Parsons (2) found that the intravenous injection of the mycelial form usually resulted in generalized and fatal histoplasmosis. The intravenous injection of the yeast form, however, regularly resulted in generalized and fatal histoplasmosis. Typical reticulo-endothelial hyperplasia with enlargement of the liver and spleen was noted, and the organisms were always found within the endothelial macrophages.

METHODS

Animals. The white mice used in this study were between the ages of 6 and 8 weeks.

Organism. The strain of *Histoplasma capsulatum* was obtained from Dr. J. D. Reid. The culture had been isolated from a patient in 1939. Stock cultures were maintained on Sabouraud's dextrose agar at room temperatures. They were transferred at intervals of 14 days. The growth was always cottony and white, and the underside of the growth never became dark before transfers were made.

The organism, when transferred to blood agar slants, sealed with paraffin and incubated at 37°C., developed as a mixture of the yeast-like and mycelial form. The growth always appeared as smooth, glistening, pale brown tenacious colonies.

A 7 day growth from these slants was ground in a mortar with sand and physiologic saline solution. The suspension was so prepared as to correspond in density to a number IV McFarland barium sulfate standard. This suspension was used immediately for inoculation.

Drugs. The drugs used were: sodium iodide; sulfanilamide; fuadin; neostam; proflavin; thymol; B-9 and sodium propionate. They were administered as sterile solutions by intraperitoneal route.

EXPERIMENTAL PROCEDURES

The general procedure was to inoculate young white mice intravenously with 0.2 cc. of a saline suspension of the organism. After inoculation they were divided into equal groups, and weighed. All groups were treated, except one, which was kept as a control. Treatment was started approximately 24 hours after inoculation. Six experiments were performed.

All mice in experiment I and II died between the 12th and 16th day after infection. Those in experiment III, IV, V and VI were sacrificed after 10 days of treatment. The liver and spleen of each mouse was cultured on Sabouraud's dextrose agar. Autopsies were performed, and sections for histologic examination were taken from liver, spleen, kidney, lung, testicle, large intestine, small intestine, head and tongue.

Experiment I. Twelve mice, 8 weeks old, average weight 18 grams (maximum 22.2; minimum 14.0), were injected intravenously with the suspension of *Histoplasma capsulatum*. The mice were then divided into 2 groups of 6 each. Group Ia received 1.8 mm. of NaI every 24 hours intraperitoneally. Group Ib received no treatment. Table 2 shows the weight of the individual animal at the beginning of the experiment, at 9 days after they were infected, and at death. From this table the duration of the infection can be determined.

Experiment II. Thirty-three mice 7 weeks old (average weight 16.0 grams) were injected intravenously with a suspension of the organisms. They were then divided into 3 groups. Group IIa (10 mice) received 0.25 cc. of 0.8% solution of sulfanilamide in saline intraperitoneally three times a day. Group IIb (10 mice) received 0.25 cc. of 1.6 Fuadin[4] intraperitoneally once a day. Group IIc (13 mice) were not treated.

[4] 1 cc. Fuadin (Winthrop) plus 5 cc. sterile water.

Experiment III. Thirty white mice 6 weeks old (average weight 18 grams) were injected intravenously with the suspension of *Histoplasma capsulatum.* They were then divided into 2 groups of 15 mice each. Group IIIa received 0.2 mgm. of neostam intraperitoneally daily for 10 days. Group IIIb received no treatment.

Experiment IV. Thirty mice 6 weeks old (average weight 16 grams) were injected intravenously with 0.2 cc. of a suspension of the organisms. They were then divided into 3 groups of 10 mice each. Group IVa received 0.05 mgm. of proflavin intraperitoneally daily for 10 days. Group IVb received 1 mgm. of thymol in olive oil intraperitoneally daily for 10 days. Group IVc received no treatment.

TABLE 2

MOUSE	WEIGHT WHEN INJECTED, 10/29	WEIGHT 9 DAYS AFTER INJECTION, 11/7	AUTOPSY	
			Weight	Date
Ia_1	18.0	20.8	20.0	11/12
Ia_2	20.7	15.7	15.5	11/10
Ia_3	19.5	21.0	20.0	11/12
Ia_4	17.0	16.9	16.5	11/12
Ia_5	21.6	19.0	18.3	11/12
Ia_6	14.0	17.5	17.5	11/12
Ib_1	22.2	25.7	23.2	11/10
Ib_2	15.5	16.9	14.0	11/11
Ib_3	16.5	15.9	—	11/11
Ib_4	20.0	22.0	22.2	11/10
Ib_5	17.5	19.8	19.6	11/10
Ib_6	20.2	21.0	21.0	11/11

This table shows the weight in grams of each mouse in group I at the time of injection, 9 days after injection, and the weight and date at the time of autopsy. The infection in this group was allowed to run its course.

Experiment V. Twenty mice 6 weeks old (average weight 16 grams) were injected intravenously with 0.2 cc. of a suspension of *Histoplasma capsulatum.* They were divided into 2 equal groups. Group Va received 1 mgm. of "B-9" intraperitoneally daily for 11 days. Group Vb received no treatment.

Experiment VI. Twenty mice 6 weeks old (average weight 15 grams) were injected intravenously with 0.2 cc. of a suspension of *Histoplasma capsulatum.* They were divided into 2 equal groups. Group VIa received 1.5 mgm. of sodium propionate intraperitoneally daily for 10 days. Group VIb received no drug.

Cultures from the livers and spleen of all mice were positive for *Histoplasma capsulatum.* The control mice, as well as the treated mice, were infected at the termination of an experiment.

TABLE 3

GROUP	NUMBER OF MICE	DRUG AND DOSAGE USED	RESULTS
Ia	6	1.8 mg. Na I every 24 hrs.	All infected
Ib	6	None	All infected
IIa	10	0.25 cc. of 0.8% sulfanilamide three times a day	All infected
IIb	10	0.25 cc. of 1:6 Fuadin every 24 hours	All infected
IIc	13	None	All infected
IIIa	15	0.2 neostam every 24 hours	All infected
IIIb	15	None	All infected
IVa	10	0.05 mgm. proflavin every 24 hours	All infected
IVb	10	1 mgm. thymol in olive oil every 24 hours	All infected
IVc	10	None	All infected
Va	10	1 mgm. "B-9"	All infected
Vb	10	None	All infected
VIa	10	1.5 mgm. sodium propionate every 24 hours	All infected
Vb	10	None	All infected

This table shows the number of mice in each group the drug and dosage used, and the results of each experiment. The drugs were all administered via the intraperitoneal route.

Table 3 shows in summary the number of mice in each group, the dosage and drug used in each group, and the results of this treatment.

AUTOPSY FINDINGS

Gross pathology. The spleens of all mice are greatly enlarged. They seem firm, and are deep

red in color. Well circumscribed pin-head, dull yellow areas can be seen frequently through the capsule. The cut surfaces are glistening, and the same small, circumscribed yellow areas can be seen. The liver is enlarged, a deep red to pale pink in color. Cut surfaces show nothing unusual. Figure 1 illustrates this point

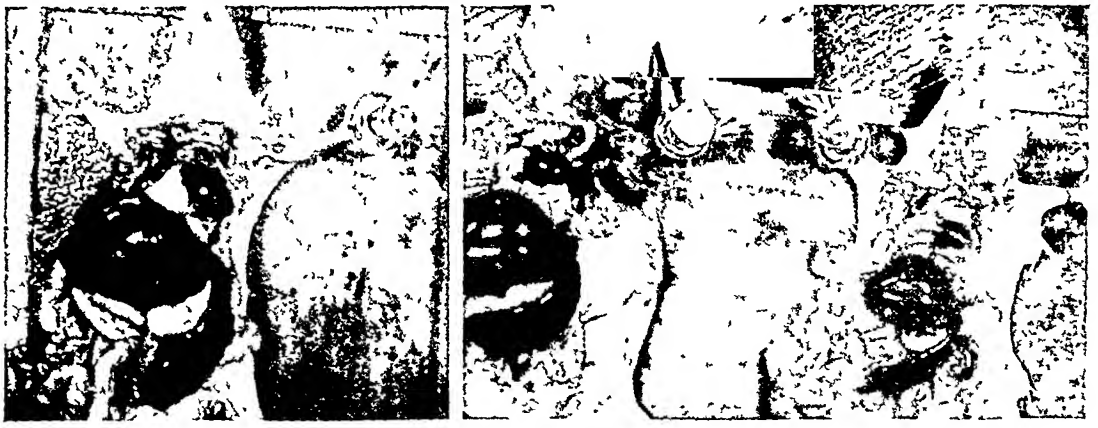

FIG 1. GROSS APPEARANCE OF THE LIVER AND SPLEEN OF INFECTED MICE. The mouse on the right is a control, normal animal

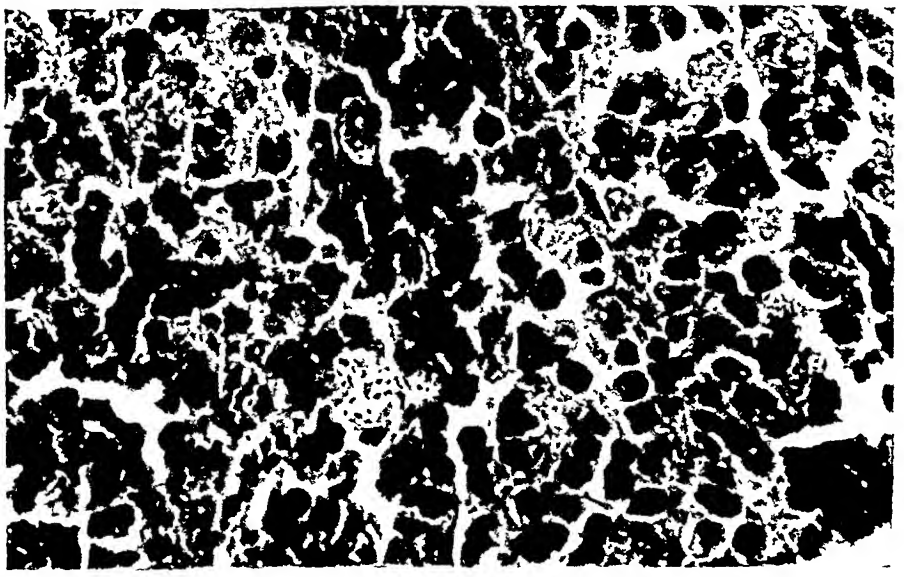

FIG 2 PHOTOMICROGRAPH OF SPLEEN SECTION SHOWING THE TYPICAL HISTOPLASMA BODIES WITHIN ENDOTHELIA CELLS
Hematoxylin and eosin (×240)

Histologic pathology

Spleen Microscopic study of the spleen in each case reveals an intact fibrous capsule The lymphoid follicles, composed of typical lymphocytes and lymphoblasts, are present in their usual number and distribution. The intervening sinusoids are lined by numerous pale staining cells whose nuclei are round and vesicular. These contain from 3 to approximately 25 small round bodies, colorless except for a slightly eosinophilic center. They are identified as phagocytic reticuloendothelial cells The ingested bodies correspond in all respects to the *Histoplasma capsulatum.* In some organs there are so many parasitized cells that they almost form solid sheets between the lymphoid follicles Scattered throughout the medulla are occasional plasma cells, polymorphonuclear and eosinophilic leukocytes. Figures 2 and 3 show typical microscopic fields

Liver. The liver cords, while still arranged in

regular manner, are compressed by large numbers of pale, mononuclear cells which lie within the sinusoids. These pale cells, in some cases, attain large dimensions and are filled with the typical Histoplasma bodies. As many as 30 bodies have been counted in some of these cells. The nuclei are vesicular, round and identical with those seen in the spleen. The number of reticulo-endothelial cells varies from animal to animal, but in each case there is a uniform distribution. In some cases there are so many reticulo-endothelial cells that it is difficult to find liver parenchyma. In other cases, there are fewer of these cells, but in no instance are they so rare that they are not immediately visible on superficial examination. Because of the presence of these reticulo-endothelial cells, the sinusoids stand out as pale, wide spaces. The cytoplasm of the liver cells proper is granular (cloudy swelling) and in areas shows the presence of small fat vacuoles. Occasional polymorphonuclear leukocytes are encountered in the portal fields and sinusoids. The portal fields are sites of lymphocytic infiltration. The central venules are dilated and filled with the same type of Histoplasma-containing cells as those described within the sinusoids. See Figures 4, 5, 6 and 7.

Lungs. The alveolae show focal consolidation. *These inflammatory lesions are scattered throughout* the parenchyma of the organ. Some are peribronchial, while others show no topographic relationship to these structures. The alveolar lumina are filled with parasitized macrophages; and occasional polymorphonuclear leukocytes, without the parasite, are noted. Within the intra-alveolar capillaries in these foci, similarly affected macrophages are seen. Many of the bronchi show a complete loss of their lining epithelium and are filled with Histoplasma-containing macrophages. The pleura in no case appears to be affected by the process, beyond minimal edema.

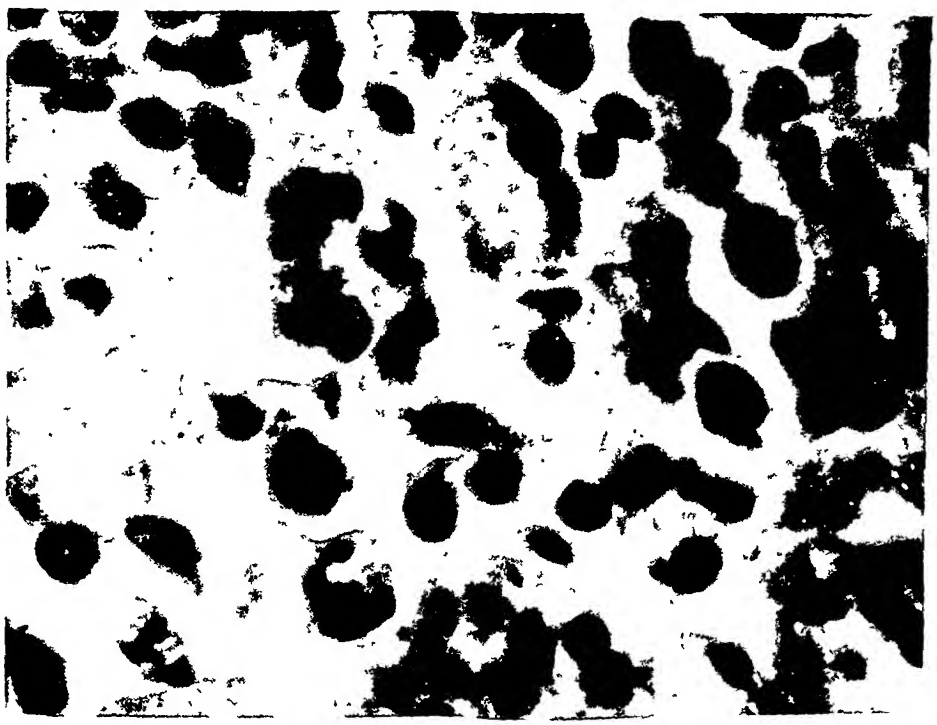

Fig. 3. Higher Power of Fig. 2 (×1240)

Kidneys. The kidneys do not show many changes attributable to histoplasmosis. *The only* constant finding is a form of interstitial nephritis. Within the connective tissue, particularly about vessels and glomeruli, small aggregates of monocytes and rare plasma cells are seen. Occasionally, within these foci, parasitized cells may be seen. No changes are seen in the medulla and pelvis of the organs.

The testes, gastro-intestinal tract and tongue show no pathologic lesions.

Six sections from one head were ready for study

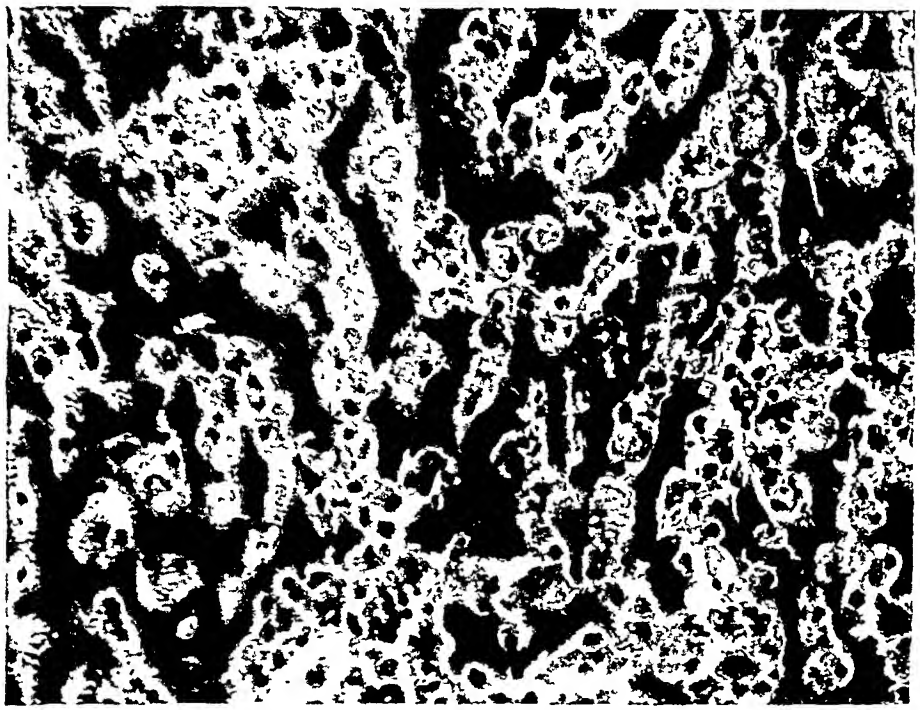

FIG. 4. PHOTOMICROGRAPH OF LIVER SECTION, SHOWING LARGE NUMBERS OF THE ORGANISM WITHIN THE RETICULO-ENDOTHELIAL CELLS (×240)

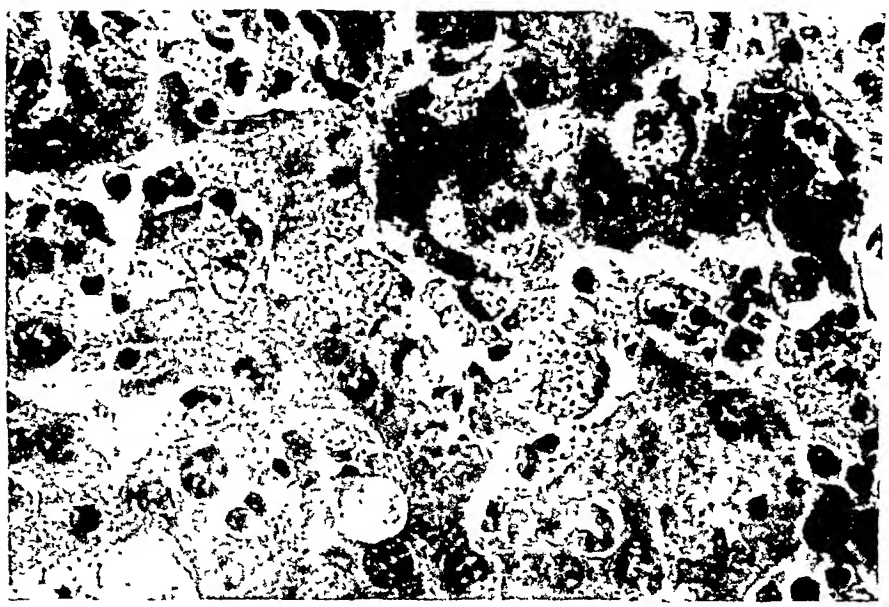

FIG. 5. HIGHER POWER OF FIG. 4 (×750)

at the time of this writing. The organisms were seen within macrophages in the incisor pulp (fig. 8), in the salivary glands, and in the brain.

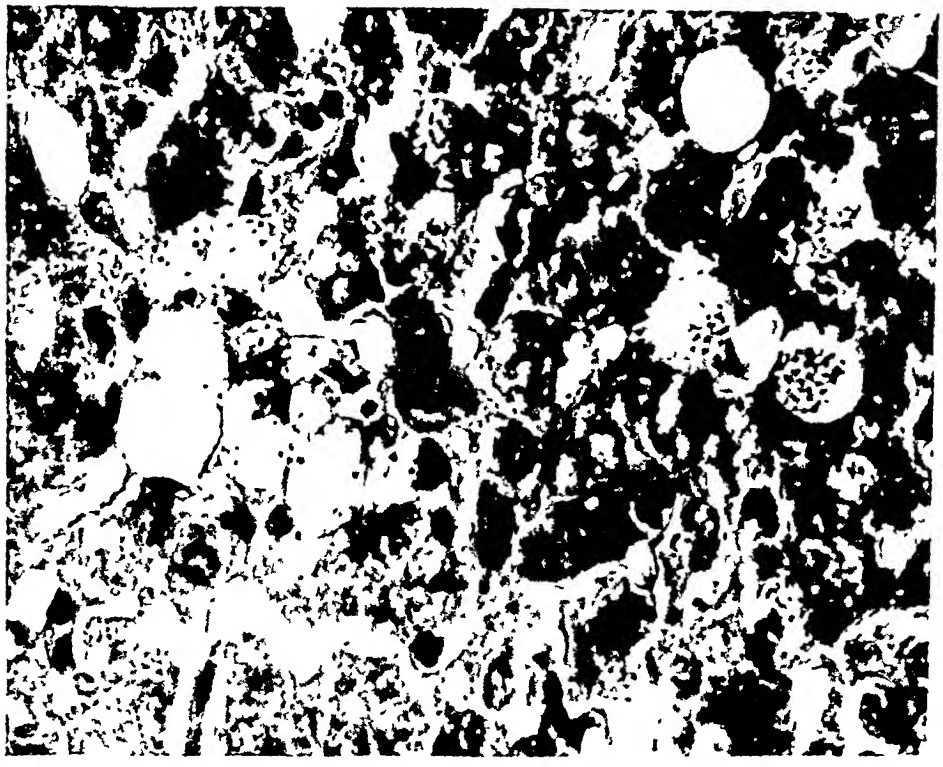

FIG 6 PHOTOMICROGRAPH ILLUSTRATING THE ORGANISM PACKED WITHIN THE KUPFFER CELLS OF THE LIVER
Note the marked destruction of the liver cells (×750)

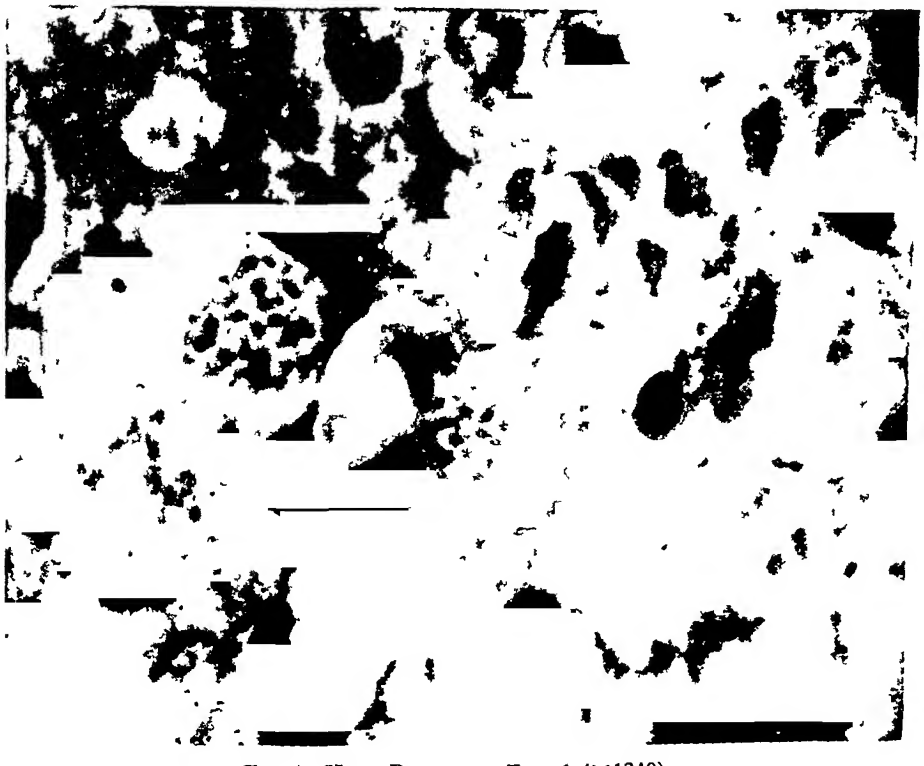

FIG 7 HIGH POWER OF FIG 6 (×1240)

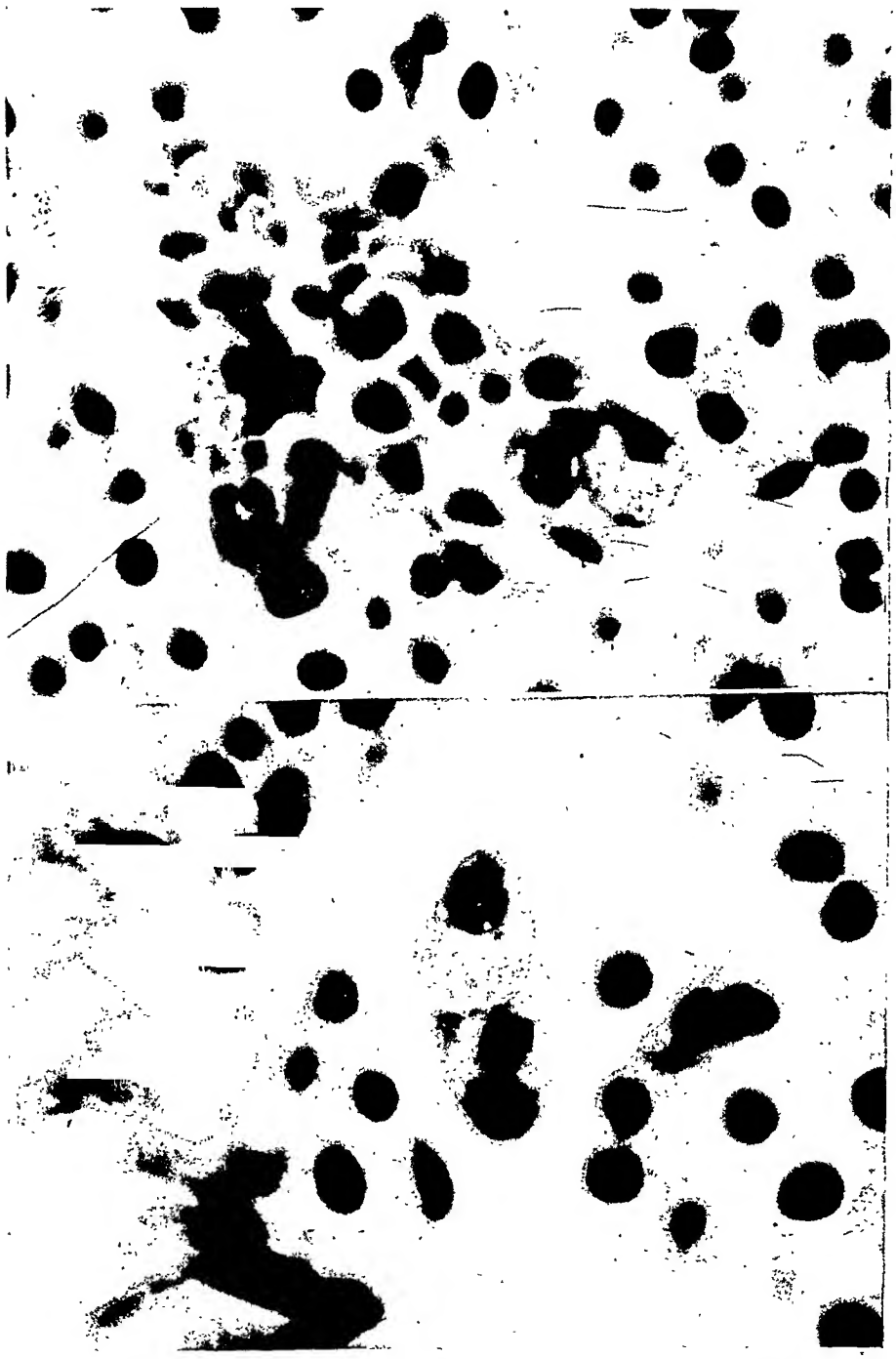

FIG. 8. The picture at the top (×1440) shows *Histoplasma capsulatum* within a macrophage in the pulp of the maxillary incisor tooth of a mouse. The lower picture (×2160) shows the parasitized organisms within a macrophage in the pulp just beneath the odontoblastic layer of cells.

DISCUSSION

The uniformity with which all mice in all groups were infected is noteworthy. However, at one time during the course of this work, the organisms lost their virulence for mice. The fungus regained its virulence only after repeated mouse passage via the intraperitoneal route. Parsons made a similar observation. The organism he was using infected all mice in a specific series. When he tried, some time later, to infect another group of mice, he found it impossible to do so. By using a growth from a slant which had been inoculated with yeast-like form he was able to infect mice at will.

All of the drugs used in this work proved to be of no value in combating the disease in mice. It is possible that in some instances the concentration of drug was not high enough to reach maximum therapeutic level. Dosages were based upon the standard dosage for human adults. Treatment may not have been carried out long enough for the drug to act therapeutically.

The fact that mice in groups III, IV, V and VI were sacrificed after 10 days of treatment may raise a question. Were our criteria for the treatment of mice adequate? We were interested in whether the drug was capable of killing the organism in vivo. Since treatment was started about 24 hours after the organisms were injected into the mouse, it appears that the fungus was capable of reproducing and infecting the animal, even in the presence of the concentration of drug used.

SUMMARY AND CONCLUSIONS

1. Intravenous inoculation of white mice with *Histoplasma capsulatum* is a satisfactory method for the study of the disease in animals, and may be used in studying the chemotherapeutic effect of drugs.

2. The drugs sodium iodide, neostam, fuadin, sulfanilamide, proflavin, thymol, B-9 and sodium propionate were ineffective in the treatment of histoplasmosis in white mice, according to our criteria.

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EXPERIMENTS TO DETERMINE POTENTIAL MOSQUITO VECTORS OF WUCHERERIA BANCROFTI IN THE CONTINENTAL UNITED STATES[1]

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The present studies were undertaken because of the prevalence of filariasis in many areas in which our military forces are operating and because of the return to the continental United States of individuals infected with this disease. It seemed desirable to have available information concerning potential vectors of the parasite in this country in order that suitable control measures may be instituted in event that conditions become favorable for the reestablishment of the disease here.

While there have been numerous investigations on the suitability of various species of mosquitoes as vectors in endemic areas, little work of this nature has been carried out in the United States, although in certain other countries investigations have included a few species found also in this country. Low (1901) working in Castries, St. Lucia, was able to demonstrate partial but not complete development of the microfilariae of *Wuchereria bancrofti* in *Culex taeniatus* (= *Aedes aegypti*); on the other hand, the larvae reached the infective stage in *Culex fatigans* (= *Culex quinquefasciatus*). Vincent (1902) showed that in Trinidad the larvae developed to the infective stage in *C. quinquefasciatus* and that development took place normally in *Anopheles albimanus*, although he was unable to keep specimens of the latter species alive until the infective stage was reached. Vincent found that in *A. aegypti* the microfilariae failed to grow beyond the sixth day. Francis (1919) demonstrated that *C. quinquefasciatus* was an efficient vector in the former endemic focus of the disease at Charleston, South Carolina, although he also was unable to incriminate *A. aegypti*.

In this country Wellman, von Adelung, and Eastman (1910) were not successful in obtaining development of the microfilariae in *Culex tarsalis* or *Culex consorbinus* (= *C. pipiens*) at Oakland, California, although Khalil, Halawani, and Hilmy (1932) found that the latter species was an efficient vector of the parasite in Egypt. In China, Hu and Yen (1933) obtained third stage infections in *Culex pipiens* var. *pallens* while Hu and Chang (1933) working in the Woosung District of Shanghai discovered infective microfilariae in 12 per cent of 245 specimens of this variety caught in houses occupied by persons suffering from filariasis. Several Japanese workers had previously found *C. pipiens* var. *pallens* to be a good vector. Yamada (1927) summarized the findings of these workers and tested this as well as additional species. He confirmed the results of previous investigators with this variety. However, *Aedes sticticus* and *A. dorsalis* failed to permit development of the larvae. In *Aedes vexans* var. *nipponii*, a close relative of our domestic *A. vexans*, Yamada found that the larvae penetrated the walls of the stomach but soon died in the body cavity.

Davis (1935) at Bahia, Brazil, tested 10 species of mosquitoes of which 5 are reported from the continental United States. A large percentage of *C. quinquefasciatus* permitted full development of the microfilariae; *C. nigripalpus* harbored occasional larvae in "advanced development"; *A. aegypti* permitted slight development followed by degeneration of the larvae; while in *A. taeniorhynchus* and *A. scapularis* no metamorphosis of the microfilariae was observed. Finally, O'Connor and Beatty (1938) found *C. quinquefasciatus* to be an efficient vector in St. Croix and observed also that *A. albimanus* was a potential vector since microfilariae developed to the third stage in this species. On the other hand, the larvae failed to reach the infective stage in *A. aegypti*.

EXPERIMENTAL METHODS

It seemed desirable to include in the testing program those species of mosquitoes whose biting habits and distribution would be conducive to

[1] Read at the Fortieth Annual Meeting of the American Society of Tropical Medicine, at St. Louis, Mo., November 13–16, 1944.

the transmission of filariasis in event that the species was found to be a favorable intermediate host for *W. bancrofti*. This program has not been completed and this paper is therefore in the nature of a progress report.

Because of the lack of suitable filariasis patients in the continental United States at the time these studies were inaugurated, the initial investigations were made in Puerto Rico with species of mosquitoes common to that island and to the continental United States. Later, the work was transferred to Alabama.[2]

Larvae and pupae of the various mosquitoes were collected in the field and bred out in the laboratory. During the course of the experiments three patients were utilized for the feeding of the mosquitoes. These patients had average counts of 42, 54, and 60 microfilariae, respectively, in 20 cmm. of blood drawn from the finger at 8:30 p.m.

Two methods of feeding were used. Those mosquitoes which were more active feeders and which appeared to be little disturbed by light and movement were placed in a lantern globe which was sealed at the bottom and covered with gauze at the top. The arm of the volunteer was placed over the gauze. The time of feeding varied between 30 minutes and 2½ hours.

Species which were more timid and would not feed well when disturbed by light, noise, or even the slightest motion were introduced into a large gauze cube supported by a wooden frame. This cage had a sleeve on one side into which the volunteer inserted his arm. In this manner, the subject could sleep during the feeding which lasted the entire night. The latter method was more convenient, but had two disadvantages. The actual period of feeding could not be determined and upon numerous occasions the subject would withdraw his arm from the cage during sleep.

Those mosquitoes that had fed were separated the next morning from those that had not fed. The engorged mosquitoes were transferred to lantern globes, dated, and maintained under conditions in which the temperature and humidity were reasonably constant. In Puerto Rico the use of pans of water and wet towels over cages maintained the temperature between 80–86°F. and the relative humidity between 70–92 percent. In Alabama the mosquitoes were kept in an insectary the temperature of which varied between 74 and 80°F. The relative humidity was maintained between 75 and 85 per cent.

The mosquitoes were observed from day to day and those that died were dissected. If dried or decomposed, they were discarded without being examined. Those that appeared incapacitated and would probably have died in a short time were killed and examined. Beginning with 9½ days after feeding, the specimens that had survived were chloroformed and dissected on successive days and the results noted.

For dissection the legs and wings were removed and the body divided into three sections, the abdomen, the thorax, and the head. These sections were teased apart in physiological saline solution under the dissecting microscope and the slide examined under a higher magnification.

Several points were taken into consideration in evaluating the condition and stage of the filariid larvae in the mosquito. First, the condition of the mosquito, whether alive or dead, was noted since the larvae seemed to deteriorate rapidly after death of the intermediate host. Second, the condition of the larvae themselves was recorded, i.e., whether dead, degenerating, encapsulated, abnormally shaped or entirely normal. Third, observations were made of the degree to which the larvae had developed in the time that had elapsed since the mosquito had fed.

In order to insure an adequate amount of data, dissections were made of at least 100 mosquitoes that had lived for 9½ days or more after feeding and were in good condition when they were killed. This time (9½ days) was the earliest at which infective stage larvae appeared in *C. quinquefasciatus*.

EXPERIMENTAL FEEDING HABITS AND DISTRIBUTION OF SPECIES EMPLOYED IN TESTS

A total of 283 feedings was made between July 1943 and October, 1944. In all, 8,198 mosquitoes were used of which 2,371 partook of a blood meal (table 1). Sixteen species have been tested to date. Of this number, conclusive evidence has been obtained for 10 species, partial evidence for 2 others, and suggestive information about the remainder. Distribution records have been taken largely from the monograph by King, Bradley, and McNeel (1944) but have been supplemented by data compiled in this Laboratory.

[2] We are greatly indebted to Dr. E. L. Bishop, Director of Health, Tennessee Valley Authority, for his courtesy in supplying laboratory facilities and for his active encouragement of the work.

Culex quinquefasciatus, the common house mosquito of the Southern United States, ordinarily bites man eagerly, but under experimental conditions required the employment of a large cage because of its timidity.

Anopheles albimanus fed quite readily although a higher percentage of feeding was obtained in the large cage than in the globe. This species was reported at one time from Key West, Florida. At the present time, it is known to occur in the continental United States only in the lower Rio Grande Valley in Texas.

TABLE 1

Feeding data on mosquitoes exposed to blood meals upon infected volunteers

SPECIES	NUMBER OF FEEDING ATTEMPTS	TOTAL NUMBER OF MOSQUITOES EXPOSED TO MEAL	TOTAL NUMBER OF MOSQUITOES FED	APPROXIMATE PER CENT FED
Puerto Rican strains				
A. sollicitans	20	200	166	83
P. confinnis	10	195	160	82
A. aegypti	14	190	132	69
A. albimanus	17	410	271	66
A. taeniorhynchus	11	349	140	40
C. nigripalpus	48	1,328	135	10
C. quinquefasciatus	7	334	31	9
C. erraticus	10	297	16	5
Alabama strains				
A. quadrimaculatus	7	112	77	69
P. discolor	6	166	101	61
P. confinnis	20	761	414	54
A. vexans	5	495	224	45
A. triseriatus	20	674	201	30
C. tarsalis	3	101	19	19
C. salinarius	20	439	72	16
A. punctipennis	54	1,801	203	11
C. restuans	8	336	0	0
P. ciliata	3	10	9	—
Totals	283	8,198	2,371	

Psorophora confinnis proved to be a voracious feeder, engorging in a few minutes in the lantern globe. Experiments were conducted with this species in both Puerto Rico and in the continental United States, where this species had been known for a long time as *P. columbiae*. The feeding habits of the species in both localities were similar, but infectivity results were so markedly different that it was necessary to consider the Puerto Rican and U. S. specimens as separate strains. The U. S. strain is rather widely distributed throughout the Southern and Southeastern States, and occurs elsewhere.

Culex nigripalpus did not feed well under the conditions prevailing in the experiments. Only 10 per cent of 1,328 specimens exposed to a blood meal engorged and many variations in handling this species were employed. It appeared that the majority of those that fed did so because of a chance contact with the volunteer's arm. This species occurs throughout the Southeastern States and is particularly abundant in certain sections in Florida.

Aedes aegypti was a willing feeder after a sufficient time had elapsed following emergence from the pupal stage. Maximum feeding occurred about 48 hours after emergence. This species occurs in most of the towns and cities of the South.

Aedes sollicitans fed quite readily and usually soon after emergence. This species occurs along the Atlantic and Gulf Coasts. It has been recorded as far west as New Mexico.

Aedes taeniorhynchus is also a voracious feeder although not usually until 48 hours after emergence. The breeding habits closely resemble the previous species. The distribution extends along the Atlantic Coast from New England to Florida and along the Gulf Coast to Mexico. It also occurs along the Southern California Coast.

Aedes vexans proved to be a ready feeder 48 hours after emergence. This species is of wide occurrence throughout the United States although it is seldom abundant in the extreme South.

Aedes triseriatus did not feed voraciously but given a sufficient length of time a fair percentage of the individuals would engorge. In order to obtain engorgement, it was necessary to starve this species before feeding. This tree-hole breeder is widely distributed throughout the United States.

Anopheles punctipennis was a rather poor feeder under laboratory conditions. It was necessary to starve the specimens before offering a blood meal. Even then, only a small percentage would feed. This species is of wide occurrence throughout the United States.

Anopheles quadrimaculatus fed quite readily under our experimental conditions. Two sources of the species were used; the majority came from an established colony while the remainder were reared from "wild" larvae secured in the field.

No difference in feeding habits was noticed. This species is quite common throughout the South and recorded from many other localities.

Culex salinarius fed rather poorly throughout the experiments. The supply of this species was meager, since it occurred but sparingly in the few places where it was found breeding. As a result, conclusive evidence for this mosquito has not been obtained. The species has a widespread distribution in the South and East.

Psorophora discolor proved to be a willing feeder. Its distribution is sparse throughout the Southern States.

Psorophora ciliata engorged eagerly, consuming a relatively large amount of blood. The species is widely distributed throughout the South, East, and parts of the Middle West, but the larvae were encountered only in small numbers.

Culex erraticus was an extremely poor feeder in tests conducted both in Puerto Rico and Alabama. A very small number fed in the earlier stage of the work, but none fed in Alabama. This species occurs throughout the Southern States.

Culex tarsalis fed in small numbers after a starvation period. Those that had been given a sugar water meal two or three days before feeding on the volunteer engorged more readily than those starved entirely. The species is common in the West and rare in the South.

Culex restuans failed to feed in the laboratory under any conditions employed. Several hundred specimens were exposed to a blood meal upon numerous occasions without success. This species is of general distribution throughout the Southern, Eastern, and Northern States.

OBSERVATIONS ON LARVAL DEVELOPMENT

The development of the microfilariae in those species in which the infective stage was reached was in conformity with the observations of other workers. Within a few hours after ingestion by the mosquito, the microfilariae had cast their sheaths and penetrated through the gut to the abdominal spaces, from which they made their way to the thoracic muscles. Here the larvae developed into the characteristic short sausage-shaped organisms. This stage was reached usually by the fifth day, although in a few specimens more time was required. This stage was followed in 2 or 3 days by the first molt. After the molt, the larva, now in the second stage, increased slightly in width and to a greater extent in length. This process continued for an additional 3 to 5 days, during which time the internal organization of the larva became more evident and material could be seen moving back and forth in the gut. The third stage, following the second molt, was marked by further elongation and a decrease in width. Two or 3 days after the second molt the mature, infective larva had evolved. Larvae in this stage were easily identified by their extreme slenderness, by the presence of 3 prominent caudal papillae, and by their violent writhing and coiling motions. After this final stage had been reached, the larva began migrating from the thorax to the head or abdomen.

In the majority of the species permitting full development, the cycle was completed within two weeks. There was some individual variation in the time required and the development within *C. quinquefasciatus* was completed on an average of two to four days sooner. One specimen of this species showed infective larvae 9½ days after feeding.

In those species permitting only incomplete or abortive development of the larvae the time schedule was irregular. Development was arrested at various stages, although the sausage stage represented the limit of development in the incompatible mosquitoes. In several species there was encapsulation of the larvae by a black, calcium-like substance which enveloped the larvae either partially or completely. This phenomenon sometimes occurred while the larvae were still alive, although encapsulated larvae were usually dead. Encapsulation was encountered to some extent in *P. confinnis* along with normal development.

In three species, *A. taeniorhynchus*, *A. vexans*, and *A. quadrimaculatus*, the larvae rarely got into the thorax. In the former two species the microfilariae apparently were digested in the gut, for rarely were there any larvae found in the rest of the body. In *A. quadrimaculatus*, very early first stage larvae were found completely or partially encapsulated in the abdominal cavity 12 to 14 hours after feeding.

RESULTS OF EXAMINATIONS

In all, 1,801 dissections were made of 15 species of mosquitoes. Detailed protocols of the experiments are given in Table 2 and the results are discussed below by species.

Culex quinquefasciatus. Twenty-seven specimens were examined, of which 17 lived 9½ days

or more after feeding. All 17 showed infective larvae at the time of dissection. These larvae were distributed at random throughout the body, the majority being in the abdomen. Nine of the 17 showed infective larvae in the head or proboscis at the time of dissection. One specimen killed after 9½ days showed 47 third stage larvae, of which 9 were infective.

Anopheles albimanus. A total of 235 of this species was dissected, of which 114 lived 9½ days or longer. Of the latter number, 40, or 35 per cent, showed infective larvae which appeared to be distributed at random throughout the body. Of the mosquitoes showing infective larvae, 24 were found to contain them in the head or proboscis at the time of dissection. Another 37, or 32 per cent, showed larvae which were developing normally at the time of dissection. These must be considered as potentially infective larvae in that they were third stage larvae that had as yet not reached the final, mature, infective stage and gave no indication of retarded development. Adding the figures for both infectives and potentially infectives, an infectibility percentage of 67 is obtained. There was very little evidence of degeneration or retardation of development in this species. On the whole, it appeared that an average of two days longer was required for the larvae to reach the infective stage than in *C. quinquefasciatus*.

Psorophora confinnis (Puerto Rico). A total of 149 specimens was dissected, of which 104 lived for as long as 9½ days. Of these, 5 showed infective larvae, one of which was a head infection, and another 8 showed potentially infective larvae. A 12 per cent infectibility rate was obtained with this species. Considerable variation was encountered in dissection results with the species. In many specimens, the larvae were surrounded by a capsule of a dark, calcium-like substance. This phenomenon was found to occur in mosquitoes which also contained normally developing larvae. There was in addition considerable variation in the degree of development attained by the various larvae found upon dissection. Mosquitoes with infective or noninfective third stage larvae often showed larvae that were still in the second stage. However, the majority of the specimens dissected were entirely negative.

Psorophora confinnis (Continental U. S.). A total of 159 specimens of this strain of the species was examined, of which 104 lived for at least 9½ days after feeding. Of these 104, 63 showed infective larvae throughout the body. Head or proboscis infection was encountered in 45 of the 63 showing infective larvae. An additional 21 mosquitoes living for 9½ days or more showed active third stage larvae which at the time of dissection had not as yet become infective. The results give an infectibility rate of 80 per cent with *P. confinnis* from the continental United States. The encapsulation phenomenon was encountered to only a slight extent in this strain. Moreover, very few complete negatives were found. While retardation of development was seen in a few specimens, on the whole development to the infective stage was accomplished in approximately two weeks.

Culex nigripalpus. A total of 128 specimens was examined, of which 102 lived 9½ days or longer. Of the latter, 3 showed infective larvae, 2 being head infections, and an additional 4 showed potentially infective larvae. These results give an infectibility rate of 7 per cent. The majority of the specimens were entirely negative. When larvae were encountered, they had usually died at the presausage or sausage stages which occur prior to the first molt. No evidence of encapsulation was found in this species.

Aedes aegypti (Including material from both Puerto Rico and the continental United States). A total of 128 specimens was examined, of which 102 had lived for at least 9½ days after feeding. Of these, 4 showed infective stage larvae and in each a head or proboscis infection was obtained. An additional specimen showed potentially infective larvae. The infectibility rate for the species was approximately 5 per cent. The great majority of the specimens showed larvae, but with few exceptions these larvae were of the very earliest first stage. For the most part, they were dead and degenerating, but occasionally, even as long as two weeks after infection, some early first stage larvae would exhibit slight motion.

Aedes sollicitans. A total of 164 specimens was dissected, of which 103 lived for 9½ days or longer. In no case was normal development encountered beyond the first or second day. Larvae were found in about half of the specimens examined. However, with the exception of an occasional presausage stage larva, they had ceased development at the early or middle first stage.

Aedes taeniorhynchus. Of this species, 136 were dissected, of which 101 had lived for 9½ days or longer. As with the preceding species, normally developing larvae were never encountered. In

fact, only 8 specimens showed any larvae and these were all in the first stage and dead.

Aedes vexans. A total of 159 of this species was examined, of which 104 lived for 9½ days or longer. The majority of the specimens were negative after the second or third day. Of the entire 159 examined, only 7 showed larvae in the thoracic muscles and these occurred singly as dead first stage larvae.

Aedes triseriatus. A total of 175 of this species was examined, of which 107 lived for 9½ days or longer. Two of the latter showed infective larvae located in the thorax in one case and in the head and proboscis in the other case. A third specimen showed a single, active, non-infective third stage larva in the thorax. However, in the majority of the specimens, the larvae encountered had died in the first stage and many were encapsulated by a black deposit. The infectibility rate of this species was approximately 3 per cent.

Anopheles punctipennis. A total of 209 specimens was examined. Of this number, 95 lived for 9½ days or more. Normal development beyond the second or third day was not encountered. In those specimens showing larvae, the latter were almost invariably dead, occasionally encapsulated, and had never developed beyond the first stage.

Psorophora discolor. A total of 41 individuals was examined, of which 11 lived for 9½ days or longer. Of the latter, one showed infective larvae and an additional 5 showed potentially infective larvae in the thorax. The one harboring infective larvae at the time of dissection did not have a head or proboscis infection. The early development of the larvae in this species was entirely normal but it appeared as though there was some retardation in the later stages. Having reached the second stage within the normal time limits, the larvae were slow in advancing to the third and final infective stages. Some showed second and third stage larvae as late as 15 and 16 days after feeding. There was some evidence of encapsulation in this species. Further information is needed.

Culex salinarius. Fifty-six specimens were examined, of which 23 had lived for 9½ days or longer. Of the latter, none showed infective larvae although a single third stage larva was found in each of two specimens. Development was irregular in that about half the specimens permitted development through the first and occasionally into the second stage, whereas in the rest of the mosquitoes development was checked early in the first stage. Encapsulation occurred rarely.

Psorophora ciliata. Very few of this species were available for study. Seven have been examined, none of which has lived longer than 8½ days. In one of these, live presausage larvae were found after 5½ days. The other 6 were completely negative.

Culex erraticus. Only 8 specimens were examined, all of which had lived for more than 9½ days. No larvae were found in the dissections.

Culex tarsalis. None of the exposed specimens has been examined at this writing.

Anopheles quadrimaculatus. Twenty specimens were examined, of which 10 had lived for 9½ days or more. All dissections were negative with the exception of one specimen killed after 12 hours. This specimen revealed several larvae in the abdominal cavity and a few in the thorax. The majority of the larvae were dead and either completely or partially encapsulated by a dark deposit.

DISCUSSION OF RESULTS

Results with *C. quinquefasciatus* in both Puerto Rico and the continental United States indicate that this species would be the most important vector of filariasis should a sufficient focus of infection be established in areas in which it is prevalent. The breeding of this species in urban and suburban areas and its high susceptibility to infection appear to make the species an ideal vector.

On the other hand, *P. confinnis*, which has shown itself to be almost as susceptible is essentially a rural breeder. It is possible that this habit would lessen the threat of this species as an important vector, at least in comparison with *C. quinquefasciatus*. Of the many localities in which *P. confinnis* adults and larvae were found, few were within town limits. The majority of breeding places were cow pastures or roadside ditches.

These factors are to a certain extent offset by the tremendous numbers in which the species emerges after a sudden flooding, the avidity with which it attacks man and its comparatively long flight range. Few residents of suburban and rural areas are safe from the attacks of this species during the height of its periodic swarmings and on occasion it may be extremely prevalent in urban communities. While from the experimental standpoint this species has shown itself to be an

excellent intermediate host, its chances of spreading *W. bancrofti* would seem to depend almost entirely upon there being a high incidence of filariasis within its flight range.

A. albimanus proved to be an efficient intermediate host experimentally. At the present time, it is known to occur only in the lower Rio Grande Valley. The breeding and feeding habits of the species are within the requirements of a good vector, but, unless it becomes more widespread, there is only a remote possibility that it might be concerned in transmission of the disease.

respect to *A. quadrimaculatus*, *C. salinarius*, *C. erraticus*, *C. tarsalis*, *P. ciliata*, and *P. discolor*. None of the exposed *C. tarsalis* has been dissected. On the basis of present information obtained with the other species, none has shown evidence of being a good host, with the possible exception of *P. discolor*. This species did permit development to the third and infective stages in some instances. However, it is felt that even should further dissections reveal a higher percentage of infection, this species would not likely be an important vector because of its apparent scarcity.

TABLE 2

Development of W. bancrofti larvae in various species of mosquitoes

SPECIES	EARLY DEVELOPMENT OF LARVAE—2 TO 9 DAYS AFTER FEEDING		LATE DEVELOPMENT OF LARVAE—9½ DAYS OR MORE AFTER FEEDING				TOTAL INFECTIBILITY PERCENTAGE	NUMBER OF INFECTIVE LARVAE PER SPECIMEN	
	Number mosquitoes dissected	Number showing normal larvae	Number mosquitoes dissected	Number showing well advanced, but not infective, larvae	Number showing infective larvae anywhere in body	Number showing infective larvae in head or proboscis		Maximum	Average
C. quinquefasciatus	10	10	17	—	17	9	17 of 17*	20	6
P. confinnis (U. S.)	55	37	104	21	63	45	80	41	6
A. albimanus	121	90	114	37	40	24	67	10	4
P. confinnis (P. R.)	45	2	104	8	5	1	12	17	5
C. nigripalpus	26	3	102	4	3	2	6.8	21	8
A. aegypti	26	1	102	1	4	4	4.9	7	4
P. discolor†	30	27	11	5	1	0	6 of 11*	3	3
A. triseriatus	68	2	107	1	2	1	2.8	2	2
C. salinarius†	33	14	23	2	0	0	2 of 23*	—	—
A. vexans	55	0	104	0	0	0	0.0	—	—
A. sollicitans	61	0	103	0	0	0	0.0	—	—
A. taeniorhynchus	35	0	101	0	0	0	0.0	—	—
A. punctipennis	114	0	95	0	0	0	0.0	—	—
A. quadrimaculatus†	10	0	10	0	0	0	0 of 10*	—	—
C. erraticus†	0	—	8	0	0	0	0 of 8*	—	—
P. ciliata	7	0	0	—	—	—	0 of 7*	—	—

* Percentages not estimated with small numbers.

† Insufficient data to estimate infectibility percentages or to draw conclusions regarding efficiency.

The low infectibility rates obtained with *A. aegypti*, *C. nigripalpus*, and *A. triseriatus* appear to rule these species out as important potential vectors. It is conceivable that in a high focus of infection any one of them might serve as a transmitter in certain instances. This is particularly true of *A. aegypti*, because of its breeding habits.

A. vexans, *A. sollicitans*, *A. taeniorhynchus*, and *A. punctipennis* would not be capable of transmitting filariasis because of their complete failure to allow normal development of the larvae.

Conclusive evidence was not obtained with

Because of the reluctance of *Culex restuans* to feed on man, no laboratory data were obtained in regard to it. However, it would appear that this characteristic in itself might rule out this species in any consideration of possible vectors.

The distribution of the infective larvae within the mosquito host has been the subject of some speculation. It has been the opinion of many that infective larvae occurring in the body of the mosquito in sites other than the head and proboscis would never be involved in the transmission. This belief has probably been based upon the supposi-

tion that normal larvae observed in the thorax and abdomen would be incapable of migrating to the head or proboscis.

In view of the fact that no arrestment other than the encapsulation of young larvae in the thorax has been observed, the authors can see no ground for this belief. The infective larvae found in the abdomen were invariably highly active and with sufficient illumination one could see them migrating about freely. Although it was impossible to trace the migration from abdomen to head, the larvae were observed entering the thoracic muscles from the abdomen and *vice versa*. Highby (1943), working with *Dipetalonema arbuta*, a filariid of the porcupine, observed the third stage larvae migrating freely from the tip of the labella to the abdomen of its mosquito host. Anatomically, there appears to be no mechanical barrier to the progress of the larvae from abdomen to head. The open-type circulatory system that is found in the mosquito would seem to afford easy transit, particularly in an anterio-posterior direction.

While it is true that the majority of the infective larvae encountered were in the abdomen, it is believed that this is purely a matter of chance, dependent upon the availability of space and the number of larvae present in an individual mosquito. Dissections delayed a few days after the infective stage had been reached showed a greater percentage of larvae in the head and proboscis than earlier dissections, indicating a general migration from the rest of the body toward this region.

On the basis of the foregoing, it is felt that the total number of infective larvae found within the body of the mosquito is more indicative of its potentiality as a vector than the number found only in the head or proboscis at the time of dissection. In many instances (table 2) infective larvae were found in the abdomen and thorax but not in the head. It is believed, however, that had dissections been made at a later date larvae would have been found in the head and proboscis in a majority of the specimens.

One particularly interesting point arises when a comparison is made of the results obtained with *P. confinnis* collected in Puerto Rico and *P. confinnis* obtained in the continental United States. Table 2 shows an infectibility rate of 12 per cent for the former and 80 per cent for the U. S. strain. An identical number of specimens of each strain was dissected, so that it is felt that the difference in results is significant. On the basis of morphology, the larvae and adults of the two types appeared to be identical. However, their susceptibility to infection with *W. bancrofti* was markedly different. It would appear from this that the species is separable into two distinct physiological types or strains, a thing which has been observed in connection with certain anopheline vectors of malaria. Further observations on strains may reveal similar differences in the capacity of other species of mosquitoes to transmit filariasis.

SUMMARY AND CONCLUSIONS

Experiments to determine possible mosquito vectors of *W. bancrofti* in the continental United States were inaugurated in Puerto Rico and later transferred to the continental United States.

Mosquito larvae collected in the field were raised to adults. The latter were permitted to feed upon volunteers infected with the periodic strain of *W. bancrofti*. The mosquitoes were dissected at various intervals after feeding and the development of the microfilariae was followed. A total of 1,801 dissections was made of mosquitoes from 15 species.

All of 17 *Culex quinquefasciatus* dissected 9½ days or more after feeding showed larvae which had developed to the infective stage.

Sixty per cent of the *Psorophora confinnis* collected in the continental United States contained infective larvae and an additional 20 per cent had well advanced larvae 9½ days after feeding. A potential infectibility rate of 80 per cent was obtained with this species.

Anopheles albimanus carried infective stage larvae in 35 per cent and well advanced larvae in 32 per cent of the specimens examined, giving an infectibility rate of 67 per cent.

Complete development of the larvae was observed occasionally in certain species which had the following infectibility rates: *Psorophora confinnis* (collected in Puerto Rico) 12 per cent; *Culex nigripalpus*, 7 per cent; *Aedes aegypti*, 5 per cent; *A. triseriatus*, 3 per cent.

No development beyond the first larval stage was observed in *Aedes sollicitans*, *A. taeniorhynchus*, *A. vexans*, and *Anopheles punctipennis*.

Inconclusive evidence has been obtained for *Anopheles quadrimaculatus*, *Culex erraticus*, *C. salinarius*, *Psorophora ciliata* and *P. discolor*. Data secured thus far indicate that none of these species is a good host, with the possible exception of *P. discolor* which permitted development to

the infective stage in one specimen with well advanced stages in five other specimens.

Development to the infective stage was attained in most mosquitoes within two weeks and followed a pattern previously described by other investigators. Head or proboscis infections developed in over half of the specimens showing infective larvae.

It is concluded that *C. quinquefasciatus*, *P. confinnis*, and *A. albimanus* are capable of serving as excellent vectors of *W. bancrofti* should the proper conditions prevail for the spread of filariasis. *C. nigripalpus*, *A. aegypti*, and *A. triseriatus* might serve as vectors although their low infectibility rates preclude their playing an important role in the spread of the disease. *A. sollicitans*, *A. taeniorhynchus*, *A. vexans*, and *A. punctipennis* are apparently incapable of transmitting the infection.

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Addendum

Since the preparation of this paper, data have become available on dissections of *Culex tarsalis*. Of 49 specimens examined 9½ days or more after feeding, 10 contained normally developing, but not infective, larvae, while 31 were found to have infective larvae; in 21 cases such larvae were found in the head and proboscis. The infective stage was usually reached in 15 days. There was no evidence of retardation of growth or encapsulation of larvae in this species. From these preliminary results, it would appear that *C. tarsalis* is potentially as efficient a vector as *C. quinquefasciatus*.

TESTS OF MERCURY AND ANTIMONY COMPOUNDS IN DIROFILARIA IMMITIS AND LITOMOSOIDES CARINII INFECTIONS[1]

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The acquisition of *Wuchereria bancrofti* infection by American troops serving in endemic areas abroad and the possibility of the reestablishment of filariasis in the continental United States have lead to a renewed interest in this disease. There is no satisfactory treatment for this condition even though numerous attempts have been made to discover some chemotherapeutic agent which would be of value. The matter of treatment involves several objectives. It is conceivable that a drug might be sufficiently effective to destroy circulating microfilariae and sterilize productive female worms without having any lethal effect on adult worms in the lymph glands or lymphatics. Such a drug might constitute an effective instrument for limiting the spread of infection or gradually controlling the disease in an endemic area although it would probably offer no immediate relief to the patient. On the other hand, it is possible that a drug sufficiently specific in its action to destroy adult worms might lead initially to exacerbations in the symptoms through the absorption of dead worms or their toxic products, even though eventually benefit might accrue to the infected individual.

A large number of investigations have been carried out on the chemotherapy of filariasis, the most recent work being that of Brown (1) who was successful in eliminating circulating microfilariae from the peripheral circulation of 2 of 11 individuals treated with relatively large doses of anthiomaline. Chopra and Sundar Rao (2) reported tests with a large number of compounds and found that Fuadin gave good results in that it caused the disappearance of microfilariae for several days and seemed to result in clinical benefit and cessation of chyluria. Hawking (3) found no microfilariae in the blood of 1 of 7 cases after treatment with Fuadin and none in 1 of 10 cases after treatment with tartar emetic. Beneficial results have been reported in *Wuchereria* infections with the use of mercury succinimide by Anderson (4) and with the use of mercury cyanide by Kingsbury (5).

For the reason that the available evidence indicated that compounds of trivalent antimony and mercury were of more promise than other types of chemical agents, preparations containing these metals were used in these experiments. For comparative purposes, certain drugs which had been used previously in filariasis were included in the present tests. The remainder consisted of compounds synthesized in our laboratories or supplied by pharmaceutical firms.

MATERIALS AND METHODS

Experimental procedure. All of the compounds were used parenterally. In the case of dogs, it was found that the intravenous route of administration was most satisfactory; for the treatment of cotton rats, the intramuscular and intraperitoneal routes were employed.

Microfilarial counts were performed by a method previously described (6) on blood samples taken at the same time each day. At least three counts were made prior to the institution of treatment, after which the counts were carried out at the time of administration of the drug. Following completion of the treatment, the animals were observed for a period of at least two months and frequently for six months, during which time microfilarial counts were made weekly. Following the period of observation, the animals were sacrificed, a search made for adult parasites, evidence of gross pathology noted, and tissues preserved for microscopical study.

Preparation of compounds. With the exception of antimony oxide, the antimony compounds were all derivatives of phenols, alpha hydroxy acids, or polyhydric alcohols.

Thirty derivatives of alpha hydroxy acids were prepared. For the preparation of these compounds, antimony oxide, tartaric acid, and the organic base (e.g., p-phenetidine) were refluxed in an aqueous solution. The volume was reduced

[1] Read at the Fortieth Annual Meeting of the American Society of Tropical Medicine, St. Louis, Mo., November 13–16, 1944.

over a steam bath and the crystalline compound precipitated out of the solution upon cooling. The antimony content, which was determined by titration after destruction of the organic material present, indicated that these compounds were the respective antimonyl tartrates. Sodium antimonyl citrate was prepared in a similar manner with the use of citric acid.

The antimony phenolic compounds included Fuadin, Stibsol, sodium antimonyl bis-catechol, and sodium antimonyl tertiary butyl catechol. The latter two compounds were prepared by refluxing an aqueous solution of the catechol, sodium hydroxide, and antimony oxide. Further separation was performed in a manner similar to that used with the alpha hydroxy acid derivatives.

It is known (7) that alkaline solutions of the polyhydric alcohols react with antimony oxide to yield water-soluble complex metal compounds; for example, sorbitol is said (8) to form a compound, corresponding in antimony content with the formula $C_6H_{13}O_5$-O-Sb(ONa)-O-$C_6H_{13}O_5$, which is known as sodium antimonyl sorbitol. We were fortunate in having available a number of polyhydric alcohols and some of their methylene derivatives and have employed twenty-four such compounds for the preparation of test solutions in the present investigation. A suspension of antimony oxide in a solution of the polyhydroxy compound in *N* sodium hydroxide was refluxed for two to four hours, the undissolved oxide was separated by filtration, and the filtrate was analyzed to determine its antimony content. It was found that the amount of antimony oxide which had reacted agreed well with the ratio of one molecular equivalent of oxide to two of the hydroxy compound. The solutions of metal complex so obtained were alkaline and in general were less stable than those of the compounds of the preceding two groups; in concentrated solutions some oxidation to pentavalent antimony compounds was noted and in dilute solutions gradual precipitation of antimony oxide was frequently noted. Efforts to crystallize the complex by precipitation with organic solvents were unsuccessful. The dosage was therefore based on the amount of trivalent antimony present in the solution. For convenience, the name of the complex was taken from the name of the polyhydroxy compound from which it was prepared; for example, the solution prepared from D-arabitol was designated as sodium antimonyl D-arabitol although upon aging it was known that some pentavalent antimony complex was present.

TREATMENT OF DOGS WITH MERCURY COMPOUNDS

Two dogs were treated with mercury cyanide at the dose rate of 0.34 and 1.0 mgm. of mercury per kilogram of body weight, respectively. The dog receiving the larger dose died after four daily injections without apparent reduction in the numbers of microfilariae or injury to the adult parasites. The other dog received 22 injections and no beneficial results were observed in spite of evidence of mercury poisoning in the animal.

One dog received 24 injections of mercury oxycyanide at the rate of 1.0 mgm. of mercury per kilogram of body weight and another dog received 11 injections of mercury succinimide at the rate of 0.7 mgm. of mercury per kilogram of body weight. Despite evidence of mercury poisoning in both animals, no changes in the microfilarial counts occurred and no evidence of injury to the adult parasites was found at autopsy six weeks later.

TREATMENT OF DOGS WITH ANTIMONY COMPOUNDS

Twenty-five trivalent antimony compounds were used in the treatment of fifty dogs. Thirty-three other compounds were synthesized but did not appear to be of special promise and were not used in the treatment of infected animals.

As the first approach to the present study, it was necessary to establish the schedule of dosage and rate of administration of the compounds which would combine the best therapeutic activity with the greatest degree of safety. This necessitated the determination of the toxicity of each compound to be tested therapeutically and entailed a great number of preliminary arbitrary plans of treatment.

The first plan followed was that of a fixed dosage given three times per week. A deficiency in this plan appeared at once for this schedule made no allowances for differences in the weights of the dogs. A schedule based upon the amount of antimony per kilogram of body weight of the animal was adopted as this gave a direct means of comparing the activity of the various agents. Administration of the drug six days per week instead of three days per week was tried and it was found that microfilariae disappeared with fewer doses. This schedule was then used for subsequent experiments.

The first arbitrary dose rate of 1.8 mgm. of antimony per kilogram of body weight was fatal to many of the treated animals. This was reduced to the dose rate of 0.8 mgm. of antimony per kilogram of body weight, the mean dosage recommended by

Wright and Underwood (9) for the use of Fuadin in *Dirofilaria immitis* infections. This rate was well tolerated and proved therapeutically effective with such compounds as Fuadin and Stibsol.

During the course of these experiments, it became evident that the therapeutic activity of any antimony compound, as measured by the disappearance of microfilariae, was not necessarily governed by the dose rate or by the total dose administered. As a matter of fact, the rapidity of treatment was found to be an important factor and it appears that this factor is undoubtedly associated with the maintenance of a certain level of antimony in the body of the animal. The following data are offered in support of this hypothesis.

Since previous work with other compounds indicated that 0.8 mgm. of antimony per kilogram of body weight daily did not produce toxic symptoms and in the case of active compounds would eradicate microfilariae from the circulation, five dogs were treated with sodium antimonyl xylitol at the rate of 0.8 mgm. per kilogram of body weight administered six days per week. These animals became microfilariae free after 6, 8, 8, 9, and 11 injections, respectively. The same dose rate with a schedule of three injections per week was tried in the treatment of one dog. It required 52 injections to free this animal of microfilariae, indicating that a certain threshold of antimony in the body must be exceeded before any toxic effect is exerted on the microfilariae and that the antimony must be given often enough to give a positive balance of intake over excretion.

Injections of 0.6 mgm. of antimony per kilogram of body weight in the form of sodium antimonyl xylitol six days per week rendered a dog microfilariae free in ten days indicating that this dosage level also falls into the active therapeutic range. A dose rate of 0.4 mgm. of antimony per kilogram of body weight of an equally active compound failed to render an animal microfilariae free after 28 injections. It may be assumed therefore that this rate of antimony intake very slightly, if at all, exceeds the excretion rate so that it is almost impossible to attain a therapeutic threshold.

Two animals were treated with sodium antimonyl xylitol at the rate of 2.4 mgm. of antimony per kilogram of body weight once a week. One animal became microfilariae free during the week after the first injection and the other dog became microfilariae free after the second injection. These results give some indication of the therapeutic level of antimony for *D. immitis* in that this level was apparently approached with a single injection at this dose rate. However, as a regular practice, such a dosage schedule is not feasible for many toxic reactions appear even though death may not supervene after such a large single injection of antimony.

The number of intravenous injections required to render dogs microfilariae free with a number of different antimony compounds is shown in figure 1. Twenty-eight of 29 dogs treated with 16 different compounds six days per week at the rate of 0.8 mgm. of antimony per kilogram of body weight were freed of microfilariae. The successful chemotherapeutic agents consisted of four alpha hydroxy acid derivatives, three phenolic derivatives, eight polyhydric alcohol derivatives, and a suspension of antimony oxide. The one unsuccessful treatment was with sodium antimonyl bis-catechol. We are aware that adequate numbers of dogs have not been treated to establish the value of any single compound but with one exception it appeared that the above-mentioned compounds given in adequate dose rates for a sufficient length of time will eradicate circulating microfilariae without regard to chemical constitution.

In only one dog has there been a recurrence of microfilariae. This dog was treated with seven doses of sodium antimonyl xylitol and the microfilariae reappeared in the peripheral circulation four months after the last injection. It is interesting to note that since the reappearance of the microfilariae there has been a daily increment of about 100 microfilariae per cc. of circulating blood. We have kept many treated animals under observation for four to eight months and in no other instance has there been a recurrence of circulating microfilariae.

During the first four or five days of successful treatment, microfilariae counts were observed to remain relatively constant or in some instances to increase. Thereafter the count dropped precipitously to zero or fell to zero over the next two or three days.

Early in the experimental study it was observed that there was a differential killing of microfilariae and of adult filariids. In untreated as well as in the treated dogs having low initial microfilarial counts, no adult parasites were generally found in the heart. However, in dogs having higher initial counts, some of the adult worms were usually found alive at autopsy even though the microfilariae had long since disappeared from the peripheral circulation.

When available, one adult female *D. immitis* was selected from each dog at autopsy for histological study to determine whether the drug employed had been responsible for any damage to the nematode tissue. The worm was wound around a short length of glass tubing and the ends fixed with small rubber bands. The cuticle was lightly pierced in several places and the preparation placed in formalin. The worm was kept on the glass rod during the period of dehydration and infiltration preparatory to imbedding and staining. This technique had the advantage that the worm was preserved in the shape of a spiral which could be embedded in paraffin and then sectioned through the axis of the coil. The stained and mounted material then consisted of a series of cross sections of the worm at 11 mm. intervals. These preparations were examined by Doctors Theodore L. Perrin and L. L. Ashburn of the Pathology Laboratory.

The evidence indicated that certain changes had taken place in the uterine contents of adult female *D. immitis* recovered from 23 dogs in which microfilariae had disappeared from the peripheral circulation. These changes did not occur in the uteri of parasites from unsuccessfully treated dogs. The exact nature of these changes will be described in a later publication.

NUMBER OF INTRAVENOUS INJECTIONS AT 0.8 Mg. Sb./Kg. OF BODY WEIGHT REQUIRED TO ELIMINATE CIRCULATING MICROFILARIAE FROM DOGS

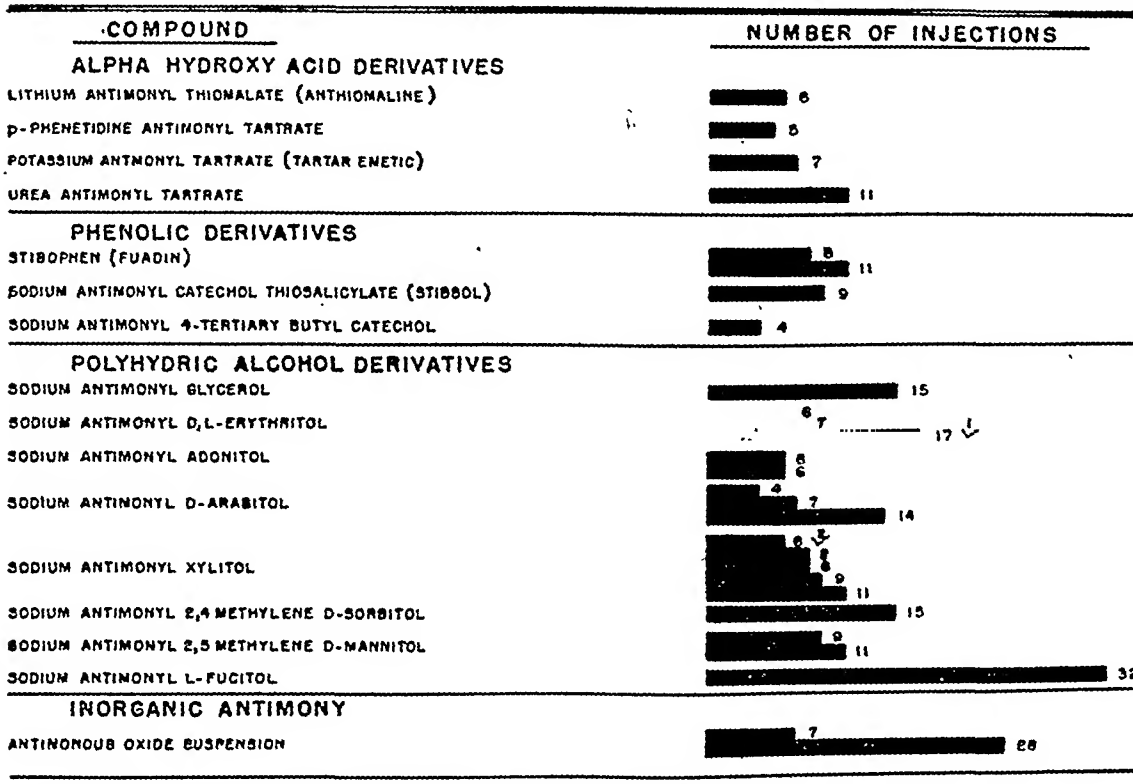

FIG. 1

TREATMENT OF COTTON RATS WITH ANTIMONY COMPOUNDS

Two infected cotton rats were treated with 40 intramuscular injections each of p-phenetidine antimonyl tartrate, p-amino-phenol antimonyl tartrate, o-phenetidine antimonyl tartrate, Stibsol, and Parke Davis and Co. No. 098429[2] respectively at the rate of 3.3 mgm. of antimony per kilogram of body weight. The adult *Litomosoides carinii* were found to have been killed when the animals were autopsied two months after the completion of the treatment. Although microfilariae were still present at the end of the period of observation, they

[2] We are indebted to Doctor L. A. Sweet of Parke, Davis and Company for his courtesy in furnishing this compound.

had decreased to one-tenth or less of their original numbers.

Two infected cotton rats were treated with 12 to 18 intraperitoneal injections each of sodium antimonyl xylitol, sodium antimonyl arabitol, sodium antimonyl adonitol, and sodium antimonyl erythritol respectively at the rate of 0.8 mgm. of antimony per kilogram of body weight. In none of these animals were there observed evidences of beneficial results from the treatment.

These results indicate that cotton rats tolerate relatively large doses of antimonyl compounds, for evidences of toxicity were not observed with the use of doses that would be fatal to dogs after several injections. During the experimental studies, it was observed that there was a differential killing of microfilariae and of adult filariids. In the case of *D. immitis*, microfilariae were eliminated with the smaller doses of various compounds without obvious injury to some of the adults. In the case of *L. carinii*, the adults were killed before the microfilariae disappeared from the circulation.

TOXICITY OF ANTIMONY COMPOUNDS

As a criterion of acute toxicity, the amount of antimony per kilogram of body weight that would kill 50 per cent of white mice within eight hours after intraperitoneal injection was determined on 32 compounds. This will be referred to as the LD 50 dose. The results of these determinations are shown in table 1 in the descending order of toxicity. It will be noted that the least toxic compound, sodium antimonyl L-fucitol, is less than one-third as toxic as the most toxic compound, urea antimonyl tartrate. This table fails to reveal any direct relationship of the chemical structure or the hydrogen ion concentration to the acute toxicity.

No correlation is revealed by a comparison of the acute toxicity of a compound and the number of injections required to free the dog of circulating microfilariae. The relationship of these two factors is shown by table 2. An arbitrary figure derived by dividing the number of treatments by the LD 50 dose, however, does give a definite indication of the comparative usefulness of these compounds. The compound having the lowest ratio is sodium antimonyl 4-tertiary butyl catechol[3]. This compound is insoluble in aqueous solutions and for injection was dissolved in propylene glycol which had the disadvantage of causing considerable local irritation. Although the next six compounds shown in table 2 have been used successfully for the

TABLE 1

The acute toxicity and the hydrogen ion concentration of various antimonyl compounds

COMPOUND	LD 50 (MG. Sb/KG. BODY WT. FOR WHITE MICE)	pH OF 0.1% AQUEOUS SOLUTION
Urea antimonyl tartrate	14.4	5.4
p-Aminophenol antimonyl tartrate	15.4	4.3
m-Phenetidine antimonyl tartrate	16.1	3.5
Lithium antimonyl thiomalate (anthiomaline)	16.5	8.7
m-Aminophenol antimonyl tartrate	16.8	3.6
Sodium antimonyl D-mannitol	16.8	11.5
Sodium antimonyl citrate	18.4	4.2
Sodium antimonyl biscatechol	18.6	7.9
Parke Davis and Co. no. 098429	18.8	8.0
Potassium antimonyl tartrate (tartar emetic)	19.3	5.2
o-Aminophenol antimonyl tartrate	19.5	—
Sodium antimonyl glycerol	19.5	11.7
o-Toluidine antimonyl tartrate	19.6	3.7
Sodium antimonyl D-arabitol	19.9	11.4
Sodium antimonyl adonitol	21.0	10.1
o-Phenetidine antimonyl tartrate	21.1	3.7
p-Toluidine antimonyl tartrate	22.1	3.6
Sodium antimonyl catechol thiosalicylate (stibsol)	23.9	6.5
Sodium antimonyl tertiary butyl catechol	24.6	7.5
p-Phenetidine antimonyl tartrate	24.9	4.8
Aniline antimonyl tartrate	26.1	3.4
p-Anisidine antimonyl tartrate	28.2	3.8
m-Anisidine antimonyl tartrate	28.3	3.4
m-Toluidine antimonyl tartrate	28.7	3.5
o-Anisidine antimonyl tartrate	29.0	3.5
Sodium antimonyl 2,4 methylene D-sorbitol	31.8	11.4
Sodium antimonyl xylitol	33.3	10.5
Sodium antimonyl erythritol	34.4	11.2
Sodium antimonyl gluco-gulo-heptitol	34.7	10.2
Stibophen (Fuadin)	35.2	6.9
Sodium antimonyl 2,5 methylene D-mannitol	44.2	11.2
Sodium antimonyl L-fucitol	46.4	11.5

[3] The 4-tertiary butyl catechol used in the synthesis of the sodium antimonyl 4-tertiary butyl catechol was furnished through the courtesy of Dr. Howard S. Mason.

treatment of 20 dogs, further investigations are necessary to determine the best compound for trial against human filariasis. These investigations must include consideration of the availability of the basic chemicals and the stability of the product.

SUMMARY AND CONCLUSIONS

Four dogs harboring *Dirofilaria immitis* were treated with three mercury compounds—mercury cyanide, mercury oxycyanide, and mercury succinimide. In spite of the fact that the dosage proved to be toxic, no therapeutic action was observed either on the microfilariae or adult worms.

TABLE 2

Ratios of toxicity and efficacy of compounds used against Dirofilaria immitis

COMPOUND	AVERAGE NUMBER OF TREATMENTS REQUIRED AT 0.8 MG. Sb/KG.	LD 50	RATIO: NO. TREATMENTS / LD 50
Sodium antimonyl tertiary butyl catechol...	4	24.6	0.16
p-Phenetidine antimonyl tartrate.....	5	24.9	0.20
Sodium antimonyl 2,5 methylene D-mannitol.	10	44.2	0.23
Sodium antimonyl xylitol.	8.4	33.3	0.25
Stibophen (Fuadin).....	9.5	35.2	0.27
Sodium antimonyl adonitol.....	6	21.0	0.29
Sodium antimonyl erythritol.....	10	34.4	0.29
Potassium antimonyl tartrate (tartar emetic)...	7	19.5	0.36
Lithium antimonyl thiomalate (anthiomaline).	6	16.5	0.36
Sodium antimonyl catechol thiosalicylate (stibsol).....	9	23.9	0.38
Sodium antimonyl D-arabitol.....	8.3	19.9	0.42
Sodium antimonyl 2,4 methylene D-sorbitol..	15	31.8	0.47
Sodium antimonyl L-fucitol.....	32	46.4	0.69
Urea antimonyl tartrate..	11	14.4	0.77
Sodium antimonyl glycerol.....	15	19.5	0.77

Preliminary investigations with trivalent antimony compounds indicated that the most favorable schedule of treatment consisted in the intravenous administration of six doses per week, each dose containing 0.8 mgm. of antimony per kilogram of body weight. With this schedule, the employment of 16 different antimonyl compounds resulted in the elimination of microfilariae from the peripheral circulation of 28 of 29 dogs infected with *D. immitis* with no recurrence during an observation period of two to six months. Some of the adult worms were killed by the treatment; changes were evident in the uterine contents of living worms recovered at autopsy.

Cotton rats infected with *Litomosoides carinii* were treated with nine antimonyl compounds. Five of these compounds in a dose rate of 3.3 mgm. of antimony per kilogram of body weight were well tolerated and were effective in killing the adult parasites. Four other compounds injected intraperitoneally at a dose rate of 0.8 mgm. of antimony per kilogram of body weight failed to have any effect on *L. carinii* although they had proved to be effective for the elimination of the microfilariae of *D. immitis* from the dog.

Comparative data on the toxicity of 32 antimonyl compounds were obtained by determination of the LD 50 dose for albino mice. It was found that the number of injections required to eliminate the microfilariae from dogs was not definitely correlated with the acute toxicity of the compound. Thus some compounds were found to have a higher therapeutic ratio than others. This difference has been indicated by dividing the number of treatments necessary for the elimination of microfilariae by a figure denoting the acute toxicity, thus providing an index which was of value in selecting the more promising compounds for further therapeutic trials.

When due consideration is given to all factors involved, of the previously untried compounds it would appear that p-phenetidine antimonyl tartrate, sodium antimonyl xylitol, sodium antimonyl 2,5 methylene D-mannitol, sodium antimonyl adonitol, and sodium antimonyl erythritol would be the most promising for chemotherapeutic experiments in human filariid infections. The last three compounds are relatively unstable in solution but means can no doubt be found for maintaining stability.

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CHEMOTHERAPY OF HUMAN FILARIASIS BY THE ADMINISTRATION OF NEOSTIBOSAN[1]

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Of the many preparations tried in the treatment of infections with *Wuchereria bancrofti* (filariasis) in man, only compounds of antimony have thus far shown much promise of success (1). Unfortunately, these antimony compounds, as used, have appeared at best to exert only a distinctly temporary effect for although circulating microfilariae have often declined in number during treatment, within a few days or weeks after treatment ended they have generally become as numerous as they were originally, before treatment began. Recently, however, Brown (2), employing a trivalent antimony preparation, lithium antimony thiomalate (anthiomaline), succeeded in eliminating all microfilariae from two patients and in sharply reducing their number in eight others among eleven blood-positive patients treated. Brown's observations covered from four to five (in one case, seven) months and revealed but small likelihood of the relapse of the infections.

Two of the present authors (3) recently showed that when neostibosan, a pentavalent antimony preparation, was injected repeatedly to filaria-infected cotton rats, the adult filarias were killed in the pleural space and the microfilariae gradually disappeared thereafter from the blood of the animals. The action of the drug appeared to be directed chiefly against the adult worms, since microfilariae continued to persist although in slowly diminishing numbers for weeks or even for months after the adult worms were dead. Because of the success which attended the administration of neostibosan in the cotton rat filaria infection, and because of the excellent tolerance man is known to have for this compound, it was decided to test the activity of the drug in persons harboring *Wuchereria bancrofti*. Arrangements were made to perform the tests in Puerto Rico, West Indies, where infection with *Wuchereria bancrofti* is relatively common. As will be seen, microfilariae have disappeared from the blood or have been reduced in number for at least six months in a majority of the patients treated.

GENERAL PROCEDURE

The patients. Of the thirty patients which were treated with neostibosan, five (Nos. 9, 11, 12, 13, and 14 of table 1) were outpatients of University Hospital in San Juan. All of the five were males who had been rejected for military service because of filariasis, and who were employed as mechanics or as water-front workmen. Of the remaining twenty-five patients, all of whom were between 8 and 17 years of age, fifteen (females) were students at Hogar Insular de Niñas, Santurce, and ten (males) were students at Hogar Insular de Niños at Guaynabo. None of the thirty patients, except No. 12, presented symptoms of filariasis; all were in apparent good health and engaged throughout the period of treatment in their usual activities as workmen or as students. Patient No. 12, who had been afflicted for several years with periodic chyluria, was brought into the hospital especially for this treatment. All of the thirty patients had microfilariae in the night blood.

[1] The authors wish to acknowledge the kindness of Dr. Pablo Morales Otero, Director of the School of Tropical Medicine, San Juan, Puerto Rico, in providing facilities for this study. They are grateful also to Dr. Ramón Suarez and to Dr. F. Hernandez Morales, of University Hospital, San Juan, for their interest in the work. Their best thanks are extended, likewise, to Dr. José Gándara, of the Insular Department of Health, who permitted treatment of certain patients in Hogar Insular de Niñas, Santurce, and Hogar Insular de Niños, Guaynabo, as well as to Dr. M. Pujadas Diaz, the Attending Physician of these institutions, who generously collaborated in the study. Finally acknowledgment is made of the helpful interest of Dr. James B. Rice, Director of Medical Research of the Winthrop Chemical Company, which supported the work.

[2] Read at the Fortieth Annual Meeting of the American Society of Tropical Medicine, at St. Louis, Mo. November 13–16, 1944.

The fifteen control patients (males) all were students at Hogar Insular de Niños at Guaynabo. None had ever presented symptoms of filariasis and all were in apparent good health. The infections in these persons were discovered during a survey made upon the bloods of all the students at the institution.

The administration of drug. During the first week of treatment, every patient was given graded doses of neostibosan, beginning usually with 50 mgm. and working up to 300 mgm. in three injections on alternate days. Thereafter, 300 mgm. of the drug were given in a single dose six days per week usually, until treatment ended in from 33 to 48 days. All injections were made intravenously.

The drug as used was well tolerated and had to be discontinued because of toxicity in none of the patients. The only distress observed was occasional nausea or emesis, these being seen within an hour after the injection of drug chiefly during the early days of treatment.

The estimation of microfilariae. The number of microfilariae in each patient was estimated by actual count of all parasites in 60 cmm. of finger blood. Blood samples were taken from every patient before treatment began and at frequent intervals, at least once weekly, during treatment.

TABLE 1

Microfilaria counts in thirty patients with filariasis treated with neostibosan

CASE NO.	AGE	SEX	WEIGHT	DRUG GIVEN	INTERVAL OF TREATMENT	NO. OF MICROFILARIAE IN 60 CMM. BLOOD FROM TREATED PATIENT AT DESIGNATED TIMES					MICROFILARIA LEVEL, % CHANGE, ENTIRE PERIOD OF OBSERVATION
						Before treatment	At end of treatment	2.5 months after end of treatment	4.5 months after end of treatment	6.0 months after end of treatment	
			lbs.	*gm.*	*days*						
1	11	F	60	7.2	40	3	0	0	0	0	−100
2	8	F	50	4.6	33	9	0	0	0	0	−100
3	10	F	48	5.8	33	24	6	0	0	0	−100
4	16	F	106	7.2	40	15	36	8	0	0	−100
5	14	F	100	7.2	40	41	36	9	2	0	−100
6	12	M	73	7 5	39	21	51	6	1	0	−100
7	17	M	134	8.1	39	27	42	27	6	0	−100
8	13	F	102	7.2	40	216	204	141	124	6	−97
9	21	M	146	7.2	38	36	12	0	1	—	−97
10	13	M	79	8.1	39	255	204	57	30	14	−94
11	26	M	112	10.5	47	150	120	66	29	10	−93
12	21	M	125	7.6	48	15	61	—	2	1	−93
13	15	F	93	6.8	40	231	207	81	48	21	−90
14	18	M	138	10.4	47	18	42	21	1	2	−88
15	12	M	87	8.1	39	6	6	3	2	1	−83
16	15	M	114	8.1	39	33	15	15	4	10	−69
17	16	F	112	7.1	40	120	126	72	60	42	−65
18	13	M	71	8.1	39	630	624	345	249	217	−65
19	13	M	76	7.5	39	297	294	171	84	111	−63
20	16	F	102	6.9	40	136	126	111	63	49	−63
21	12	F	74	6.5	40	27	54	18	9	12	−55
22	16	F	85	6.0	40	154	129	138	123	81	−47
23	13	M	76	8.1	40	72	120	72	46	39	−43
24	11	M	58	6.9	39	177	96	114	64	111	−37
25	14	F	138	7.2	40	129	156	84	76	87	−32
26	14	F	91	7.1	40	78	87	90	31	56	−28
27	11	M	56	7.3	33	18	9	9	2	15	−19
28	8	F	52	6.4	33	123	93	90	129	109	−11
29	14	F	140	7.0	40	216	255	180	150	199	−7
30	16	F	118	6.2	33	54	66	66	51	121	+124

Similar blood samples were obtained, always at precisely the same hour of night, two and one-half, four and one-half, and six months after treatment ended. From the control untreated patients, similar blood samples were procured at intervals over seven and one-half months, always, for a given patient, at the same hour every night. The blood films were dehemoglobinized in distilled water, fixed in methyl alcohol and ether (50% each), stained with Bullard's hematoxylin, partially destained in 0.5 per cent alcohol, and washed in water prior to examination. The numbers of microfilariae shown in tables 1 and 2 represent the actual numbers seen and counted in 60 cmm. of each patient's blood by searching the entire film under 100x magnification.

TABLE 2

Microfilaria counts in untreated control patients with filariasis

PATIENT NO.	AGE	NUMBER OF MICROFILARIAE IN 60 CMM. FROM BLOOD FROM UNTREATED PATIENTS AT DESIGNATED TIMES				MICROFILARIA LEVEL, % CHANGE, ENTIRE PERIOD OF OBSERVATION
		When first observed	After 1.0 month	After 6.0 month	After 7.5 month	
1	14	360	303	318	333	−7.5
2	13	45	21	27	45	0
3	12	309	—	383	343	+11
4	14	63	3	22	76	+20
5	14	135	153	162	203	+50
6	12	315	285	333	481	+52
7	12	27	31	36	45	+66
8	17	27	—	31	54	+100
9	14	42	66	52	87	+107
10	16	3	6	6	7	+133
11	14	6	6	9	15	+150
12	14	9	6	13	25	+177
13	9	87	—	175	247	+183
14	14	27	31	55	106	+292
15	8	3	—	15	30	+900

Sex: all are males.

RESULTS

By the end of the period of treatment, microfilariae had disappeared from the blood of only two patients. Both of these persons prior to treatment had had only very low microfilaria counts. Among the remaining twenty-eight treated patients, no very sharp reduction in microfilaria levels was observed, and, in many, parasite counts at the end of the period of treatment were somewhat higher than they had been before treatment began. During the six months after treatment ended, however, microfilaria levels gradually declined in most of the treated patients. In fifteen of the thirty patients, these counts declined at least 80 per cent and from seven of these fifteen persons, all of the parasites evidently had disappeared. In only one of the thirty treated patients did the microfilaria level fail to decline. The microfilaria counts and other relevant data on the thirty treated patients are given in table 1.

Among the fifteen control untreated patients the parasite counts declined in only one individual and remained unchanged in another over a period of seven and one-half months. Of the remaining thirteen controls, all showed a rise in parasite level, and in eight of the thirteen persons this rise amounted to at least 100 per cent of the initial count. Furthermore, eight of the control patients showed an increase in the number of parasites at each successive sampling over the seven and one-half months of observation. The microfilaria counts and other data on the fifteen untreated control patients are given in table 2.

DISCUSSION

The authors appreciate the considerable limitations of the results obtained in this work. It is obvious that no reduction or only insignificant change occurred in the microfilaria levels in 25 per cent of the patients treated. Yet it can be pointed out that parasite counts declined more than 40 per cent in all others—i.e., in 75 per cent of the treated patients. From only seven patients, and these with relatively low initial microfilaria levels, have all microfilariae evidently disappeared by the sixth month after treatment ended, yet by this time eleven others—including some of those with the highest initial parasite counts—reached their lowest microfilaria levels for the period of observation and were still evidently declining. In contrast, the parasite levels in all but two of the fifteen control untreated patients rose steadily during this interval.

The general rise in the microfilaria counts of the control patients requires comment. The authors believe this rise due to the fact that the control patients, like most of the treated patients, were young and had probably harbored their infections for a relatively brief period before first observation. Consequently, microfilariae probably were still accumulating in the blood of the untreated persons. The decline in microfilariae among the treated persons, then, assumes added significance.

One of the unusual observations in this work was the tardiness with which the effects of treatment manifested themselves, a significant drop in the microfilaria counts not occurring until weeks after the treatment ended. If the results previously observed in the treatment of cotton rats (3) can be carried over to the human infection, however, it may well be that the drug affects or kills chiefly the adult parasites and that the microfilariae disappear from the blood in their own good time only after the adult worms are dead, and independently of direct effects of the antimony.

It should be pointed out, finally, that these patients were not treated intensively. The usual daily dose of neostibosan, which is recommended by the manufacturer for treating kala azar, was employed. Treatment was discontinued because of toxic effects of the drug in not a single patient. All but one case (no. 12, with chyluria) were engaged in their usual activities during the whole of the period of treatment. It is our belief that much better results than are presented in this paper could be obtained if the amount of neostibosan given were substantially increased, or else administered over a briefer period.

SUMMARY AND CONCLUSION

Thirty patients with filariasis (*Wuchereria bancrofti*) were treated with neostibosan for intervals ranging from 33 to 48 days. By the sixth month after treatment ended, microfilariae had disappeared from seven of the patients and had declined in all but one individual. In fifteen of the thirty treated patients, over 80 per cent of the microfilariae were lost during the six months of observation.

Among fifteen control untreated patients with filariasis, followed for the same period as those under treatment, thirteen persons showed an increase in microfilariae, one showed a small decline, and one presented no change in the number of circulating parasites.

Neostibosan has, therefore, appeared to exert a significant effect as a therapeutic agent in cases of filariasis *bancrofti*. It is impossible, however, as yet, to determine whether this effect is permanent, or subject to eventual relapse.

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PENICILLIN TREATMENT OF EARLY YAWS

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Following the report of Mahoney, Arnold and Harris (1) on the treatment of early syphilis with penicillin it seemed of interest to determine the effect of this drug on the closely allied spirochetal disease, yaws. This report is a preliminary note on the results of treatment of five cases of early yaws.

METHOD

The patients were chosen from a group of Melanesians who came to a native hospital in the New Guinea area for treatment of their yaws. Syphilis had never been observed in this native group. In order further to exclude the possibility of their having syphilis, only young patients with what was considered to be typical yaws were chosen for study. They received no treatment other than the penicillin.

Darkfield examinations of the lesions were carried out prior to treatment and on the day after treatment was begun. In each case several preparations were made and examined by two observers. Specimens of blood were obtained before treatment and two and three weeks after beginning treatment. The standard Kahn qualitative test was done on each specimen.

DOSAGE

Case 1 received 250,000 units of penicillin given in doses of 25,000 units intramuscularly every 4 hours. The other 4 cases each received 400,000 units given in doses of 20,000 units 5 times daily at 4 hour intervals.

CASE REPORTS

Case 1, a 15 year old girl, had a single, rounded, nodular lesion 2 cm. in diameter on the right elbow. Darkfield examination of this lesion showed very numerous *Tarponema pertenue.* Fifteen hours after starting treatment no spiral forms could be found. The lesion healed well, being considerably smaller in one week, and at 3 weeks only a very small healed nodule was present.

Case 2, a boy age 6, had a small raspberry lesion 1.5 cm. in diameter on the nose and a large, thick, secondarily infected lesion on the buttock measuring 8 cm. in diameter. Darkfield examination of the nasal lesion was positive. The darkfield examination was repeated 24 hours after treatment was begun and no spiral forms could be found. The nasal lesion healed well, and in 9 days only a small depigmented area was visible at the site of the lesion. The large lesion on the buttock looked much cleaner and drier within two days and healed rapidly thereafter. At 3 weeks it was almost completely healed.

Case 3, a boy age 4, had a flat condylomatous type of lesion 6 cm. in diameter in the intergluteal region. The lesion was a large, raised plaque, with a glistening, moist, reddish surface. It was darkfield positive before treatment and negative 24 hours after starting treatment. After about 48 hours the lesion appeared dry except in one small area in the center, and there was definite evidence of healing at the periphery. Following this there was further drying of the lesion and by the second and third weeks marked healing had occurred.

Case 4 and 5, age 1 and 4 years respectively, had lesions almost identical with that of Case 3, and were thought to be typical yaws. Several darkfield examinations of these lesions were negative. In both cases the healing of the lesions was good and proceeded at about the same rate as in Case 3.

SEROLOGY

All cases had a positive (4 plus) Kahn reaction before treatment and also at the second and third weeks after starting treatment. An attempt was made to follow the serologic reactions for a longer period, but this was unsuccessful as the natives always disappeared when the local lesions had been cured.

COMMENT

Two criteria were available in evaluating the efficacy of penicillin in these cases: first, the disappearance of the surface organisms, and second the healing of the local lesions. In the first instance the change from a positive to a negative darkfield within 24 hours in 3 cases indicates a favorable response and is comparable with the

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results obtained in the treatment of early syphilis with arsenical drugs and with penicillin. As far as the second criterion is concerned the rate of healing compared favorably with that we observed in numerous similar cases of yaws treated with relatively large doses of neoarsphenamine every 5 days. This type of yaws lesions when untreated is said to last from 3 months to 2 or 3 years (2). Spontaneous healing would thus not have been expected in this short period of observation.

SUMMARY

Five cases of early yaws were treated with penicillin. The drug caused healing of local lesions in all five cases that was comparable to the favorable effect usually obtained with arsenical drugs. In 3 darkfield positive cases the drug caused prompt disappearance of the surface organisms. It is felt that further experimental trial of penicillin is indicated in this disease.

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SURVIVAL TIME OF TROPHOZOITES OF ENDAMOEBA HISTOLYTICA AND ITS PRACTICAL SIGNIFICANCE IN DIAGNOSIS[1]

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In the diagnosis of acute amebic dysentery, it is most essential to secure a freshly voided stool for the detection and identification of trophozoites of *Endamoeba histolytica*. However, the question is frequently raised as to whether such a sample of stool should be left at room temperature, or in a refrigerator, or in an incubator prior to examination. In view of the possibility that a delay in examination may occur for one reason or another, and that an additional sample of stool may not be obtainable, the question is one worthy of careful consideration. Just how long trophozoites of *Endamoeba histolytica* ordinarily live in fecal matter outside of the body is still to be determined. This paper embodies the results obtained in an attempt to reach a workable solution. It is hoped that these findings may serve as an aid to those engaged in the diagnostic procedures.

METHODS

Among cases of clinical amebiasis which came under our observation recently, the following six cases were chosen, as the stools were typical of acute amebic dysentery and harbored only trophozoites of *Endamoeba histolytica*. Variable proportions of the amebae ingested red blood cells in each instance. No cysts were found.

Each stool received for examination was divided into approximately three equal portions and placed in clean tightly covered containers. They were then exposed to varying environmental temperatures, i.e., room temperature varying from 22°–25°C., incubator temperature at 37°C., and refrigerator temperature at 5°C. No attempts were made to modify the consistency or reaction of the stool during the course of the experiment. Microscopical examination of a bit of stool taken from each sample was made at varying time intervals, and the survival time of the trophozoites determined chiefly on the basis of motility. Culture method (1) as well as permanent stained technic, were utilized to substantiate the findings whenever deemed necessary.

A small portion of dysenteric stool from each case was inoculated into the culture medium. Luxuriant growth of the organisms usually occurred after several transfers were made at every two days interval. These culture amebae were similarly exposed to the varying temperatures as above and the behavior of the trophozoites studied in order to determine any variation that might be induced by the cultivation of the organisms.

TABLE 1

Survival time of trophozoites of E. histolytica at varying temperatures

CASE NO.	STRAIN	ROOM TEMPERATURE (22°–25°C.)	REFRIGERATOR TEMPERATURE (5°C.)	INCUBATOR TEMPERATURE (37° C.)
		hours	*hours*	*hours*
1	C.R.	16	96*	5
2	W.W.	8	72	3
3	R.A.	6	48	2
4	E.V.	8	96	3
5	J.G.	10	72	4
6	G.T.	12	72	5

* One sample—9 days.

OBSERVATIONS

It was found that among the strains of *Endamoeba histolytica* studied, there seemed to exist differences in temperature tolerance as indicated in table 1.

In looking over table 1, we may say that the trophozoites of *Endamoeba histolytica* were found to survive the longest time at the refrigerator temperature and the shortest time at incubator temperature. The time of survival at room temperature was intermediate. Thus, in these strains, the survival time of the trophozoites ranged from 48 to 96 hours at refrigerator temperature; 6 to 16 hours at room temperature, and 2 to 5 hours at incubator temperature. Though the number of amebae exposed to these varying temperatures rapidly decreased from hour to hour,

[1] Read at the Fortieth Annual Meeting of the American Society of Tropical Medicine, at St. Louis, Missouri, November 14–16, 1944.

the few which survived showed no apparent sign of degeneration. They were able to maintain the pseudopodial extrusion, though lacking in the characteristic directional motility. This was induced, however, in most instances by examining the material in a warm chamber, or by the use of a substage lamp which provides necessary warmth. In one of the specimens (case 1), it is interesting to observe that erythrocytes were still present within a few amebae that survived as long as nine days at refrigerator temperature, and showed no evidence of hemolysis. Upon application of the warmth as described, there was a more or less constant agitation of the red blood cells within the amebae and this was later followed by explosive extrusion of ectoplasm, first in one direction and then in the other. Following a number of such pseudopodial extrusions, alternated with retractions, some of the amebae assumed a somewhat elongated shape and exhibited a slowly progressive motility. In none of these amebae were we able to observe the progressive motility characterizing the amebae in freshly voided stools. However, in all instances, the viability of the amebae was verified by the use of culture medium, since seemingly quiescent organisms were often found to be able to multiply and exhibited the characteristic motility following cultivation.

As the time of exposure became prolonged, the number of the amebae was gradually decreased until finally none could be found microscopically or by culture. Upon exposure to the incubator temperature, the amebae appeared to undergo more rapid degeneration. As a rule, they are able to survive no longer than a few hours. This may be explained by the detrimental effect of the ever-increasing bacterial population and the accumulation of their metabolic bi-products.

Simultaneously with a rapidly decreasing population of the amebae from day to day, some of them became degenerated by shrinkage of the protoplasm leading to a complete disintegration or else appeared as if "baked" or "mummified." The latter were the types occasionally met with when the stool was left standing in an incubator for a prolonged period of time.

In this study no attempts were made to determine the survival time of the trophozoites of *E. histolytica* under conditions of strict anaerobiasis, nor was a buffer solution, which might modify the longevity of the parasites, added to the stool. Studies along this line are now in progress.

It is known that the morphology of degenerating trophozoite of *E. histolytica* in stained preparation may often be mistaken for the trophozoite of *Endamoeba coli*. Craig (2) describes it as possessing the thicker nuclear membrane, irregularly massed peripheral chromatin granules and larger karyosome, together with considerable chromatins in the nuclear interspace. In evaluating the suitability of a stool sample for definite diagnosis in the event of delay in examination, the above factor was taken into consideration along with the motility of the organisms, either natural or induced, and the extent of visibility of the nucleus. Furthermore, the cultivability of the organisms was determined particularly in the absence of the characteristic motility and of red blood cells within the amebae. On the basis of these evidences we may say in general that an accurate identification can best be attainable by immediate examination of a freshly voided stool. However, should a delay in examination occur, the specimen may be allowed to stand at room temperature up to three hours with no apparent loss of characteristic morphology necessary for definite diagnosis. If examination can not be made within this time, the specimen may be placed in a refrigerator but it should never be placed in an incubator at any time. In case the typical directional motility is not observable, the diagnosis should await the outcome of culture study including the examination of permanent stained preparation of culture amebae.

The survival time of culture amebae, similarly exposed to varying temperatures as above, presents a somewhat different picture. Irrespective of the source of the organisms, the behavior of the amebae, once grown in culture, apparently exhibits essentially the same degree of temperature tolerance. Thus, they were able to survive for 2 days at room temperature and 3 days at refrigerator temperature. As contrasted with the brief period of survival of trophozoites in the original stool when exposed to incubator temperature, some of the culture amebae were able to maintain viability for as long as four days. However, one should not lose sight of the fact that not only does the culture provide necessary nutriment for the growth of the amebae, but a certain proportion of the organisms has undergone encystment during the course of cultivation.

In the culture medium, following more or less a prolonged period of incubation at 37°C., mummified amebae were frequently encountered. Mummification may also occur in various tissue cells as the result of degeneration. It is suggested, therefore, that repeated examinations of stools

may become necessary to rule out the possibility of amebae being responsible for the presence of such forms in the culture medium.

Rivas (3) was probably the first to study the effect of temperature upon *E. histolytica* and reported that low temperature, instead of detrimental was found to prolong the life of the parasite. Thus, upon exposure to outdoor temperature at −5°C., the amebae were able to survive over 24 hours. The present report amply supports Rivas' findings and stresses further the importance of proper handling of stools of acute amebic dysentery in the event of delay in examination or when an additional sample of stool can not be obtained.

COMMENT

Though a majority of free-living and parasitic protozoa live within a comparatively narrow range of variations in temperature, the degree of tolerance tends to vary among different species. Various strains of the same species may likewise respond differently to temperature as exemplified in this experiment. Among factors which are responsible for the variation we may cite the extent of bacterial population and growth under varying temperatures. Equally significant is the variation in the composition and reaction of the medium as well as the degree of desiccation resulting from exposure.

Zinsser and Bayne-Jones (4) are of the opinion that bacteria of the colon group may develop at temperature as low as 10°C. and as high as 40°C. or over and that in acid medium, the death of the organisms occurs more readily than in alkaline medium. In general, the stools of amebic dysentery are usually acid in reaction. This may partly explain why some of the strains were able to survive longer in dysenteric stool due probably to the death of some of the accompanying bacteria.

On the basis of survival time of trophozoites of *Endamoeba histolytica* as determined in this experiment, it is suggested that irrespective of the type of stool, culture methods should be utilized in order to ascertain whether the few which survived are capable of bringing about the growth and multiplication sufficient for definite diagnosis. It has been our experience that such attempts often resulted positively. The author (5) observed that culture amebae devoid of starch granules within them may at times present a somewhat shadowy appearance with an apparent loss of motility. Hence they were designated by him as "hungry amebae." These, however, may be reactivated by an addition of rice starch to the culture medium. A seemingly negative finding may therefore be converted into a positive one by the treatment.

Of further interest is the observation, not reported in this paper, that trophozoites of a strain of *Trichomonas hominis* were found to survive as long as 21 days at 5°C. and 15 days at 22°C. Upon exposure to 37°C. the organisms were able to multiply profusely only for the first few hours but disappeared shortly afterwards. Tsuchiya and Jean (6) reported that the detection of the organisms was facilitated by first emulsifying a sample of stool with physiologic saline solution and by examining the sediment of the tube following a few hours' incubation at 37°C.

SUMMARY

1. Survival time of trophozoites of *Endamoeba histolytica* was determined upon exposure to varying environmental temperatures i.e., 5°C., 22°–25°C., and 37°C. and results reported.

2. On the basis of data obtained an attempt was made to reach a solution whereby we may be able to determine the duration of time and temperature at which a sample of stool can be left prior to examination without losing the characteristic morphology necessary for definite diagnosis.

3. Immediate examination of freshly passed stool is the prime requisite in the diagnosis of clinical amebiasis. However, the finding reported in this paper may serve as an aid in diagnosis in case there is a delay in examination or when an additional sample of stool may not be obtainable.

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ICHTHYOTOXISM—FISH POISONING

A Report and a Review

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Poisoning by an inherent fish toxin is described by Walker (4) as "ichthyotoxism." In the West Indies area outbreaks of this type of fish poisoning have been reported by Walker (4), O'Neill (3), Mann (5) and Gilman (6) particularly in Puerto Rico and the Virgin Islands. Poisoning of man by fish may occur in two ways; 1) as a form of food poisoning either by a toxin arising from decomposition or by toxins present in the living fish and 2) by inoculation of toxins through wounds inflicted by the fish.

This report is concerned with two outbreaks of fish poisoning occurring during the months of November and December, 1944, in Honolulu, Hawaii following the eating of varieties of sea bass (species *Variola louti* and *Serranus fascoguttatus*) from Midway and Christmas Islands in the South Pacific. The writers believe that, in each of these outbreaks involving known 24 and 14 cases respectively, the fish poisoning was due to the presence of inherent toxin in the living fish. From all available information and evidence, the fishes were brought in to Honolulu under adequate and satisfactory refrigeration conditions on ships with such facilities. All persons who brought the fish in and those who prepared and consumed them reported on the apparent visible and olfactory freshness of the fish. Portions of the fish that caused the outbreaks were examined by the Health Department's bacteriologist. The Food Commissioner, the writers and others commented on the apparent "freshness" of the fish. One old Japanese man in the second outbreak ate portions of the fish raw and became ill within two hours. In one family portions of fish were eaten by their dog, which also became ill. Several families reported illness of their pet cats and dogs following the consumption of this fish. The writers therefore believe that these two outbreaks were caused by a toxin, probably an alkaloid, present in the living fish and that this poisoning in man is similar to that reported in the West Indies area. Other fishes brought in from these same areas at the same time did not cause any illness following their consumption.

David Starr Jordan (1), in the chapter on Adaptations of Fishes, from his textbook, "A Guide to the Study of Fishes," states that in certain groups of fishes a strange form of self-protection is acquired by the presence in the body of poisonous alkaloids, by means of which the enemies of the species are destroyed in the death of the individual devoured. Such alkaloids are present in the globefishes or puffers, the filefishes and in some related forms. The alkaloids produce a disease known as Ciguatera (a term popularly used for fish poisoning in the West Indies) characterized by paralysis and gastric derangements. Severe cases of Ciguatera with humans, as well as with lower animals may end fatally in a short time.

He states that in certain groups of fishes in the tropics, individual fishes are sometimes rendered poisonous by feeding on poisonous mussels, holothurians or possibly polyps, specimens which at certain times and especially in the spawning season develop alkaloids which may cause Ciguatera in man. The true cases of Ciguatera are produced by the specific poisonous alkaloids most highly developed in the globefishes or puffers. These alkaloids are most developed in the ovaries and testes and in the spawning season. They are also found in the liver and sometimes elsewhere by the body of the fish.

The illness produced by these alkaloids must be differentiated from that produced by the leucomaines and the so called ptomaines in decaying flesh or in the oil diffused through it. Poisonous bacteria may be destroyed by cooking but the alkaloids which cause Ciguatera are unaltered by heat.

EPIDEMIOLOGY

On November 27, 1944, Dr. H. Q. Pang, one of the authors of this article, reported to the Health Department that he had treated individuals for

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an illness that was characterized by vomiting, diarrhea, severe aches and pains in the extremities and the feeling of numbness and tingling of the hands and feet, which followed the eating of fish brought in from Midway. Investigation revealed two dozen cases all giving a similar history of being given fish brought by a friend who had come from Midway.

A catch of sea bass, red snapper and mahimahi was brought in to Honolulu Wednesday evening, November 22, 1944, and distributed to friends. In most instances the fish were cleaned and kept under refrigeration until November 27 because of the "Thanksgiving turkey." On Monday, November 27, the person who brought in the fish, although having being warned by his friends that the fish he had brought back from Midway made them ill, went ahead and had a gathering of eight people, all of whom suffered from an attack of fish poisoning within four to six hours. He and his wife felt that the portions of the fish he had brought back looked and smelt so fresh that he was sure they were not poisonous.

A sample of the fish was brought in to the Board of Health laboratory and from the general appearance, the bass did look "fresh." Bacteriological examinations did not reveal any organisms on culture and direct smears. All patients who ate the fish identified the causal agent as the sea bass.

With the assistance of Mr. Vernon E. Brock, ichthyologist for the Board of Agriculture and Forestry of the Territory of Hawaii, the fish was identified as a sea bass, species *Variola louti* and found in the waters of Christmas, Palmyra, Wake, Samoa, Society, Fanning, and Apiang Islands and the waters near the Red Sea. It has nine spines on the dorsal fin, is handsomely colored scarlet or carmine with many rose colored spots on its body, and a characteristic yellow-strip marking on its dorsal, anal and caudal fins.

The other species of sea bass that was brought in from Christmas Island that was identified by patients as the causal agent belongs to the group *Serranus fascoguttatus* (Fowler). It was commonly described "black sea bass" by the patients who received the fish and is found in the waters of Palmyra, Ponape, Gilbert, Marshall, Christmas, Tongo Reva and Fanning Islands. It is dark olive in color, rather pale with brown round spots and with 12 spines on the dorsal fin.

Jordan (1) and Tinker (2) classify this fish in the Sea Bass or Grouper family (includes the sea perch tattlers and barbers) and technically the species called "*Epinephelus*." Tinker (2) describes this species as the family of the sea bass in a larger family of carnivorous marine fishes. There are more than 400 known species in this family. Among the Hawaiians it is known as the "Hapapuu"; the Chinese as "Shakpan"; and the Japanese as "Ara." One of the patients who had been stationed at Palmyra said that the Navy Department, during the period he was there, had signs out warning the personnel about eating fish caught in the lagoons during certain months of the year. The red snapper was implicated. Dr. Peter Buck of the Bishop Museum (personal communication) said that in his classes to the service personnel assigned to the areas in the South Seas, that the natives should be questioned about the possibilities of "poisonous fishes" in those areas. In the Honolulu Star Bulletin on July 12, 1944, a news item indicated that one Sergeant L. O. Shermer of the Altadena substation had been given leave of absence to catalog poisonous fishes for the foreign economic administration. The navy handbook mentions only three poisonous fishes but Mr. Shermer states that there are literally hundreds of fishes containing a poisonous alkaloid so deadly that only one mouthful would be fatal.

CASE HISTORIES

The following are case histories of patients as reported and seen by Dr. H. Q. Pang in the first outbreak. In the second outbreak the Health Department fortunately was able to warn all persons receiving portions of the fish immediately so that only 14 known cases were reported. The signs and symptoms were similar to those reported in the first outbreak except that the symptoms came earlier. Some of the patients became ill within two hours after eating the fish. In one family a one year old child became ill following the eating of fish soup prepared from the sea bass.

Case 1. G. K., a white male, age 50, ate some black sea bass at 7:30 p.m. on November 26, 1944 and at 12:30 the next morning began having diarrhea which was accompanied by slight abdominal pains. Stools were liquid and yellow in color but later were bile stained. He had about ten to twelve bowel movements at ten to fifteen minute intervals. There was no nausea or vomiting. At 2:00 a.m. his lips began to feel numb, followed in about fifteen minutes by numbness on both sides of his face and then both hands. At the same time he began having aching pains in both thighs and knees not accompanied by any rigidity.

There was no pain below the knees. Pain increased in intensity till 8:00 a.m. and codeine had to be administered for relief.

He also noticed a peculiar burning sensation in his lips and mouth while drinking a glass of water. A cold object or a glass of tap water at room temperature felt like "dry ice" in his palms. This sensation was present in the plantar surfaces of his feet and he found it difficult walking barefooted because of this peculiar feeling. The dorsal surfaces of his hands felt normal. Chest, heart and abdomen were normal. Knee jerks were present.

Two days later he began having pruritis of both palms and plantar surfaces of his feet but there was no visible rash. His testicles felt big and heavy although there were no visible or palpable signs of enlargement or edema. He had to force himself in urinating as urine did not seem to flow freely.

He was hospitalized on November 29, 1944; because of pains, irritability and insomnia. His urine revealed a slight trace of albumin. Blood count showed an 85% HB., 4,800,000 RBC., 8,500 WBC., 76% Polys, 19% small lymphocytes and 5% large lymphocytes. One hundred milligrams Vitamin B, intravenously, was administered daily without any relief of pain or paresthesia.

Codeine by mouth gave temporary relief and had to be repeated. He was discharged from the hospital on December 5th, still complaining of paresthesia in his palms and soles but was free from pain.

Case 2. Mrs. E. W., Chinese female, age 34, attended the same party as case 1 and ate the same type of fish. Diarrhea began at 12:30 a.m. on November 27, 1944 but she was free from abdominal pains or colic. She had three liquid stools at five minute intervals. Her lips and face began to feel numb, which she described as "a feeling I get after novocaine injection for tooth extraction." Her thighs and legs began to ache with increasing intensity and her back felt as if she was being tied in a knot. Pruritis of the palms and soles began on November 29, 1944. She also complained about the same "dry ice sensation" in her lips, mouth and hands as in case 1. Physical examination was essentially negative. The urine showed a slight trace of albumin. Calcium gluconate 10 cc of 10% solution was given intravenously with almost immediate relief of pain although muscular tenderness and paresthesia remained. Relief from pain, however, was only temporary and codeine grain ½ and aspirin grain 10 had to be supplemented. She received four daily injections of calcium gluconate. One week later muscular aches and pains were completely relieved with the exception of a slight residual backache. The "dry ice" sensation was present up to 10 days.

Case 3. R. L., Chinese male, age 28, ate some black sea bass at 6:00 p.m., November 24, 1944. He began having vomiting and diarrhea with only slight abdominal pains at 10:30 p.m. the same day. The next day he felt weak and began having sharp shooting pains in his hands, arms, legs and feet. He described pain as being alternating, first in one leg and then in the other. He began complaining of pruritis on November 27, 1944, which lasted till December 3, 1944. Pruritis was localized to his abdomen, below his knees, feet and hands. He also had the same peculiar feeling in his hands and mouth as in case 1. His description of drinking a glass of tap water was vivid, "I couldn't swallow a glass of cold water fast enough because it burnt my mouth." This abnormal sensation was absent in the oesophagus or stomach.

Case 4. Mrs. R. L., Chinese female, age 24, wife of case 3, ate the same fish at the same time. Her diarrhea was more severe than in case ±3 but subsequent symptoms were almost the same.

Cases 5–7. Mr. and Mrs. F. A. and daughter, Miss E. A., Chinese, ate some black sea bass on November 25, 1944 at 8:00 p.m. At 1:30 a.m., November 26, 1944, Mrs. F. A. began to have chills and her arms and legs began feeling numb. At 2:00 a.m. she began having pains all over her body, especially the joints. However, she did not have any nausea, vomiting or diarrhea. I saw her at 5:30 a.m. and at this time she felt weak and somewhat prostrated and complained of severe pains in her arms and legs. She was unable to walk a few steps without pain. Paresthesia was already present and she described a numb feeling in her fingertips and toes. Codeine grain ½ and aspirin grains 10 relieved the pains but had to be repeated every 2 or 3 hours. Improvement and disappearance of symptoms were gradual and on December 8, 1944 she felt well but slightly weak. Knee jerks were absent on further examination on November 28, 1944.

At 12:30 a.m. on November 26, 1944, Mr. F. A. had about six liquid stools at five to ten minute intervals and his chief complaints were pains in both knees and thighs. Numbness of his hands were only slight but he noticed weakness of his bladder and had to force in urinating as in case 1. He was able to be up and about on November 27, 1944 although he still had muscular aches and pains and pruritis of his palms.

Miss E. A. had severe vomiting spells and about six watery stools. At 5:30 a.m. when I examined her she seemed to be cold and clammy and was complaining of body aches and pains. Heart, lungs and abdomen were normal. On November 27, 1944, a papulo-macular eruption associated with itching appeared on her chest, abdomen, arms and thighs, which lasted for two days. Calamine lotion relieved the itching.

Case 8. Miss A. C., Caucasian, age 26, ate some black sea bass on November 27, 1944, and had vomiting and diarrhea spells followed by muscular aches, pains

and tingling sensations in her fingertips. Five others in the same party also became ill four to five hours after eating this fish. These cases were diagnosed as ptomaine poisoning by their own physician.

DISCUSSION

No studies of the suspected fish have been made and limited facts are available about the clinical studies of the victims, but because no fatalities have been reported, no pathologic or post mortem studies exist. Outbreaks in the West Indies usually occurred during the months of August through January. This report discusses fish poisoning caused by the eating of varieties of fish brought in from the warmer waters of the South Pacific. It will be difficult to determine from the fish brought in whether they are toxic to man. Certain months of the year when the algae, plankton or mussels that these varieties of fish may feed on should be known so that the public can be warned about the toxic qualities of them. If the fishing industry expands to these atolls in the South Pacific, more cases of this variety of fish poisoning will occur. The problem will continue to be difficult to control if more fish is brought in from the South Pacific until we know the varieties of fishes that are poisonous, the time of the year they are edible or poisonous and the locality where the poisonous varieties of fishes are caught.

Fish poisoning due to an inherent toxin causing similar outbreaks have been reported in the literature, particularly in the West Indies area. Walker (4), Mann (5), and Gilman (6) reported in detail the outbreaks in Puerto Rico and the Virgin Islands. Patients reported in those outbreaks presented identical signs and symptoms as in the Honolulu outbreak. The same pathognomonic signs and symptoms characterized by vomiting, diarrhea, aches and pains in the joints and extremities, followed by tingling and numbness of the extremities and the feeling of coldness when warm objects are touched as contrasted to the feeling of warmth when cold objects are touched, followed the eating of apparently fresh fish. In this series gastrointestinal symptoms have short duration in comparison to nerve symptoms. Diarrhea was for only half a day or a few hours while nerve symptoms lasted for days.

Gilman (6) in his review on this subject considered the symptoms of fish poisoning as clear cut and pathognomonic. The onset is from one to six hours after eating the fish. The patient appears critically ill with severe gastrointestinal symptoms of nausea, vomiting and diarrhea. There is a distinct metallic taste (this was not noted in the Honolulu outbreak). The skin becomes flushed and there is tingling and itching that may last for days. Cramps in the extremities may occur. Subsequently there may be reduced or absent knee jerks. Hyperesthesia and paresthesia are commonly associated. There may be associated nervousness, restlessness or insomnia; albumin, casts, and frequency of urination are prominent symptoms. The course may run from one to three weeks with gradual recovery. No fatalities have been recorded.

Walker (4) and Gudger (7) in their papers describe as possible causes for the formation of inherent toxins in the living fish the eating of poisonous "manchineel," (fruit of the West Indies area) molluscs, zoophytes, corals, medusae, holothurians and protozoans containing a toxin which may be harmless to fishes. In the literature the toxin is described to be a toxalbumin (an alkaloid) present in certain living forms in the sea.

Steinbach (8) in his article on fish poisoning in the Marshall Islands in 1893 reported that the natives recognized at that time that certain varieties of fish were toxic to man when caught during some months of the year and in specified areas. The symptoms described by him were headache, nausea, diarrhea, fever at times, and cold hands and feet combined with prickling and numbness.

Of interest in the literature is the statement of natives and writers that the larger the fish of the particular specimen that may be a carrier of the toxin, the more dangerous and toxic it is to man. The relation to spawning seasons and infectious diseases in the fish are described as possibilities as causes of poisoning in man. It is also known in the literature that in certain localities dangerous fish caught in one part may be eaten with impunity while others caught barely a mile away are regarded as poisonous.

Hermann Somner and K. F. Meyer, in the California State Department of Health Weekly Bulletin on April 26, 1941, discuss in detail mussel poisoning, a paralytic form of shellfish poisoning. It is a severe form of food intoxication caused by eating mussels or clams caught off the California coast, particularly at certain times of the year. The State Health Department issues an annual quarantine of mussel or clam fishing during the months of May through November. Up to July 1940, 310 cases of mussel poisoning with seven

deaths and 20 cases of clam poisoning with five deaths were reported in California. The original source of the poison here is a plankton which occurs most abundantly in the summer. When they multiply to such an extent as to total 40 million per liter, the water for miles presents a deep red color and at night a beautiful luminescent spectacle. The poison is an alkaloid belonging to the class such as strychnine, muscarine and aconitine and has been purified to a high degree in the form of its hydrochloride. Tested on mice, it has been found that one millionth of a gram is sufficient to kill the animal. The fatal dose for man is probably a few milligrams. It is heat stable in acid or neutral solution but is gradually destroyed by boiling with alkali. The mussels ingest the plankton and store the poison in their digestive glands without harm to the mussel. The resulting toxicity is proportional to the number of plankton ingested and to their alkaloid content. The stored poison is slowly excreted by the mussel in a few weeks.

Fish poisoning caused by alkaloids in the puffer fish has been reported frequently in the literature and many studies and investigations have been made on this form of poisoning, particularly in Japan and the Philippines. In these outbreaks the fatalities have been high.

SUMMARY AND CONCLUSION

Thirty-eight known cases of fish poisoning, in two separate outbreaks, caused by the eating of varieties of sea bass from Midway and Christmas Islands of the South Pacific, are reported. It is believed that the etiologic agent is an alkaloid present in the apparently fresh fish which is heat stable. The signs, symptoms and public health implications are discussed.

The authors recommend that studies and observations should be made by those who have the facilities and trained personnel to classify, study, experiment with and record dangerous fishes that may produce fish poisoning in waters of the South Pacific and the Hawaiian area. Physicians and others in this area should be on the lookout for this form of fish poisoning and should report cases or suspected cases to the Health Department as soon as possible to prevent the development of additional cases.

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Stitt's Diagnosis, Prevention and Treatment of Tropical Diseases. Seventh Edition. By RICHARD P. STRONG, M.D., D.Sc., D.S.M., C.B., Professor of Tropical Medicine, Emeritus, Harvard University Director of Tropical Medicine, Army Medical School, Colonel, M.C., U. S. Army. Two volumes, pp. i-xvi, 1-1747. The Blakiston Company, Philadelphia.

In July, 1942, the reviewer had the pleasure of reviewing the sixth edition of this work and at that time stated that he was convinced "that it is the most up-to-date and valuable work upon the subject that is available." And this new edition, called for in less than three years, is even an improvement over the sixth edition, and still merits the statement formally made that "it is a great work upon tropical medicine and will long remain a classic upon the subject."

The new edition has been brought up-to-date in this rapidly advancing subject and should serve as not only a fine work for the medical officer and practitioner in this country and in the subtropical and tropical regions in which our troops are operating, but, because of its encyclopedic character, should serve as a most valuable reference work upon all the subjects discussed. In these days, when almost every month or so a new book upon tropical medicine appears, it is a relief to find in this great work practically all that is known regarding tropical diseases, as many of the new books upon the subject are limited in their information and sometimes inaccurate.

The reviewer can cordially recommend this book in every respect. The volumes are beautifully bound and printed and the illustrations are many and excellent

CHAS. F. CRAIG.

Etiology, Diagnosis, and Treatment of Amebiasis. CHARLES FRANKLIN CRAIG, M.D., M.A. (Hon.), F.A.C.S., F.A.C.P., Colonel, United States Army, Retired, D.S.M., Late Commandant, Army Medical School, and Assistant Commandant, Army Medical Center, Washington, D. C., Emeritus Professor of Tropical Medicine, Medical School, Tulane University of Louisiana, New Orleans, La. Pp. v-viii, 1-332, figs. 1-45. The Williams & Wilkins Company, Baltimore, 1944.

After studying this book the reviewer is impressed by the fact that no author has previously undertaken such a careful and complete consideration and study of what is known of amebiasis at the present time as has Colonel Craig. In this book he has written not only of his important personal observations and investigations carried on in the past forty years but he has also made a careful study of the literature and has incorporated references to the important investigations made by other observers; thus it is not only an important source of information on all aspects of the subject but its value is enhanced as a book of reference.

In his preface Colonel Craig refers to the fact that in 1934 he published his work entitled "Amebiasis and Amebic Dysentery" which included the salient data available to that date upon this important subject. However, during the past decade, since that work was published, an enormous amount of research has been accomplished upon almost every phase of amebiasis and in this new book he has included all the important data accumulated during that period adding it to the material published in the previous work. He also points out that despite the many publications upon amebiasis that have appeared during the past decade, there is a surprising amount of ignorance regarding this infection among many physicians as evidenced by the letters received by him requesting information upon various phases of the subject. This, he believes, has been due to the general belief that amebiasis is a tropical infection of little interest to physicians in temperate regions, a belief that is very erroneous and harmful. At the present time thousands of our troops are serving in regions where amebiasis is a common and often serious infection and many of these men will return to the United States infected with *Endameba histolytica*, the cause of amebiasis. This will add to the already considerable percentage of infections with this parasite in this country, conservatively estimated at ten per cent of the population, and will render the diagnosis and proper treatment of the infection of still greater importance from the standpoint of public health. For these reasons this book should prove of great value to the general practitioner, public health official, and medical officers of the Army, Navy and Public Health Service, as he has endeavored to include in it all of the data of value now available regarding amebiasis.

The volume is divided into twelve chapters and there are a list of references and separate author and subject indices. Thoroughly considered are the Etiology, Epidemiology, Pathology, Symptomatology, Complications and Sequelae, Clinical Diagnosis, Laboratory Diagnosis (Microscopical and Cultural), Laboratory Diagnosis (Serological), Prognosis and Prophylaxis, and Treatment. The scope of the book covers all phases of the subject and is written in a clear and concise manner.

In Chapter I following the definition of amebiasis and amebic dysentery the history and geographical distribution are considered. The author calls attention to the fact that it is most unfortunate that the term "amebic dysentery" should have become in the minds

of most medical men a synonym of amebiasis or amebic infection for while dysenteric symptoms are quite characteristic of the serious infections with *Endameba histolytica* the vast majority of such infections are not accompanied by dysenteric symptoms but by much milder symptoms usually attributed to some other factor and not recognized as a result of infection with this parasite. This general use of the term "amebic dysentery" was caused largely by the fact that amebic infection was considered of tropical origin and in the tropics dysentery is very frequently one of the symptoms of amebiasis. The recognition of the fact that amebic dysentery is but a small part of the clinical picture of amebiasis is essential to any intelligent understanding of infections with *Endameba histolytica* and he writes it is most encouraging to note that almost all modern writers have abandoned the term "amebic dysentery" in describing such infections and have adopted the general term "amebiasis" as was recommended by Colonel Craig many years ago. He explains that the modern clinical conception of amebiasis as distinguished from the phase known as "amebic dysentery" has been a gradual growth and little had been written upon the subject prior to a paper written by him and published in 1921, calling attention to the fact that amebic dysentery is in this country a comparatively rare condition but that diarrheas and other symptoms of gastro-intestinal irritation are very commonly caused by *Endameba histolytica* even in presumably healthy "carriers" of this parasite. This contribution stimulated much interest in the subject and today he believes all well informed physicians recognize that amebiasis is responsible for many clinical symptoms far different from those of acute or chronic amebic dysentery. The recognition of the true character of amebiasis has been a great step in advance in its history and has led to the institution of proper methods of prophylaxis and treatment.

In the consideration of the geographical distribution which is world wide, it is noted that while infections are more numerous in the tropics and subtropics, in poorly sanitated districts in temperate and cold countries the incidence may be very high. Thus in the United States cases of amebic dysentery have been reported from practically every state in the Union and there can be no doubt that many cases are wrongly diagnosed annually because of the widespread belief that this type of dysentery occurs only in the tropics or subtropics. While amebic dysentery is most frequently observed in the southern states, cases of it have been noted in the most northern ones and that it may occur in epidemic form in the northern states during the summer months was well demonstrated in the Chicago outbreak of amebic dysentery in 1933.

In Chapter II the etiology is carefully considered in detail. In the discussion of the morphology of *Endameba histolytica* the four stages in its life cycle—the vegetative, motile or trophozoite stage; the precystic stage; the cystic stage; and the metacystic stage—are all considered in detail and it is pointed out that in each of these stages the organism varies somewhat in morphology. The differentiation of the pre-cystic forms of *Endameba histolytica* from those of *Endameba coli* is often extremely difficult and may be impossible. In the discussion of the life cycle of *Endameba histolytica* it is remarked that it is still a disputed question as to just what occurs after emergence of the four-nucleated ameba from the cyst and that the question of how many amebae are produced from each cyst is still uncertain and that some authorities have observed the excystment of amebae of this species containing one and two nuclei only, rather than the four-nucleated ameba as has been more commonly described. The author believes there is no evidence sufficient to demonstrate the division of the nucleus in this species as mitotic in character but rather that it is intermediate between mitosis and amitosis.

The epidemiology is considered in Chapter III. In the discussion of the methods of transmission from person to person through food or drink polluted with feces containing the parasite, it is noted that as a general rule only the cysts are infective; but that nevertheless, it has been proved that infection may occur from swallowing the motile trophozoites although these are usually destroyed by the acid secretion of the stomach. The reviewer concurs in this opinion; Weselmann and Ott have independently found trophozoites in duodenal contents removed by duodenal sound but there is no evidence that their presence here was not due to excystment somewhere in the intestine. Craig explains that patients suffering from acute amebic diarrhea or dysentery are not usually concerned in the transmission of the infection as cysts do not usually occur in diarrheal or dysenteric stools. It is when the stools become more normal that the individual is likely to become a cyst passer or carrier of the infection and that he is especially a source of infection to others.

He remarks that infection occurs especially through a polluted water supply, and that contamination by sewage of a local water supply may cause severe outbreaks of amebic dysentery. In many tropical and subtropical countries human excreta is commonly used for fertilization of vegetable gardens, and vegetables from gardens fertilized in this manner and eaten uncooked are a very common source of infection. The infection of man from food and drink contaminated by apparently healthy foodhandlers, that is, carriers or cyst passers, is regarded by Colonel Craig as probably the most common method in otherwise well sanitated towns and cities. The contamination of food and drink by the droppings of flies or of cockroaches carrying the cysts of *Endameba histolytica* also is sometimes important as a method of transmission. Finally, he explains that direct contact in the transmission of amebiasis is possibly of much importance under certain conditions as in insane asylums, orphanges and other

public institutions where hygienic standards are low and infection, especially of children, has resulted from the transference of cysts from one to another by contact with contaminated objects or material in and about the home; cysts having been found upon the hands and soiled underclothing of the children especially. Another source of transmission noted may be through the public swimming pool where the amount of chlorine generally used in the water is insufficient to kill the cysts.

The resistance of the cysts of *Endameba histolytica* to various physical and chemical agents is discussed and it is pointed out that it is now well established that the usual amounts of chlorine employed in water purification for the destruction of disease-producing bacteria are not efficacious for the destruction of the cysts of *Endameba histolytica* and that it is necessary to overchlorinate the water and then dechlorinate it if this chemical is to be used for rendering contaminated water harmless; however practical methods have been devised for rendering water safe in the field for troops by efficient hyperchlorinating and then dechlorinating. The use of iodine compounds, particularly for the destruction of cysts in individual water supplies and canteens, is not referred to. Apparently the reports of the demonstration of the effective amebacidal action of bursoline tablets, which contain iodine and diglycine hydriodide and dihydrogen pyrophosphate have been published since the book was written.

It is also noted that precipitation and filtration through sand filtration beds is satisfactory for the elimination of the cysts. However, in general travelers in regions where amebiasis is prevalent should always insist upon using only boiled water or bottled water from non-endemic regions and food should also be protected from cockroaches as well as flies.

Virulent and avirulent strains of *Endameba histolytica* are considered in detail and the important researches of Meleney and Fry and others are discussed.

It is pointed out that while amebic dysentery almost always occurs in the form of sporadic cases, under certain favorable conditions it may occur in epidemic form, and the author describes such epidemics observed by himself in the Philippines and in Texas, and records the two epidemics which occurred in Chicago during 1933 and in 1934. Evidence is given demonstrating that *Endameba histolytica* may be a parasite of some of the lower animals especially monkeys, rats and occasionally dogs. However, just how important that may be as reservoirs of infection for man is not yet clear.

In Chapter IV upon the pathology, the absence of clinical manifestations in individuals who nevertheless may have more or less advanced lesions in the intestine is discussed at some length. In regard to a very recent study of the pathology in carriers, the observations of Faust (1941) are cited. Faust studied the findings at autopsy and made microscopic examinations of sections of the bowel in 202 individuals who were accidentally killed in New Orleans, and found lesions containing the ameba in 13, or 6.44%.

The pathology of amebic ulceration of the skin is discussed in detail and the observations of Meleney are quoted to the effect that the largest number of ulcerations occur after drainage of an amebic abscess; and that ulceration of the skin usually occurs about one week after surgical drainage of an abscess has been started and that the ulceration extends rapidly and is very painful. Amebic granulomata occurring in the large intestine are also mentioned and it is pointed out that the masses may closely resemble carcinomata and may be very easily confused with the latter. Donald and Brown (1940) have reported two cases of amebic granuloma of the rectum.

In Chapter VII in the discussion of the complications and sequelae amebic appendicitis is considered and the author writes that it should not be forgotten that many of the cases so diagnosed clinically as appendicitis do not have any inflammation of the appendix but suffer from symptoms simulating those of appendicitis. Nevertheless, in a considerable proportion of fatal cases of amebic dysentery the appendix presents marked evidence microscopically of inflammation, and amebic ulcerations are present within the organ. He cautions that in every individual presenting symptoms of appendicitis an examination of the feces for *Endameba histolytica* should be made and if found positive anti-amebic treatment should be tried before resorting to surgical measures unless the symptoms are such as to demand immediate operation.

In Chapter VIII the clinical diagnosis of the disease is fully discussed. In regard to the Roentgen ray in the diagnosis of intestinal amebiasis, the observations of Manson-Bahr (1940) are quoted to the effect that x-ray diagnosis has been tried out on an extended scale at the Hospital for Tropical Diseases, London; occasionally filling defects are observed in the cecum, but similar appearances are seen in other forms of dysentery and colitis, and it is disappointing to record that only unsatisfactory assistance can be obtained by this method. However, Craig explains there is no question of the great value of Roentgen ray examinations in the diagnosis of amebic abscess of the liver and that Ochsner and DeBakey in their series of amebic abscesses of the liver obtained a positive diagnosis in 132 of 150 cases, or 88%, in which this method was employed.

In Chapter IX laboratory diagnosis, both microscopical and cultural, is considered and the practical value of the complement fixation test is discussed. This test, as devised by Colonel Craig, follows that usually employed in the Wassermann test for syphilis, using extracts of *Endameba histolytica* as antigens. In his test a human hemolytic serum is employed instead of the sheep system and Craig believes that more accurate results are obtained with the human system. This test was described by him first in 1929 and the results obtained with it are such as to convince him

that none of the modifications since published are more accurate or useful than this original technique which is again published in his "Laboratory Diagnosis of Protozoan Diseases," 1942.

In Chapter XII treatment is fully discussed and it is pointed out that at the present time we possess several drugs that are practically specific. *Emetine hydrochloride* is first discussed and its amebacidal[1] properties emphasized. It is pointed out, however, that the drug is toxic and that when used in the treatment of dysentery or severe diarrhea it should be used only long enough to control the diarrheal or dysenteric symptoms and should never be given for a longer period than eight to ten days in a dose of 0.065 gram (1 grain) a day, administered subcutaneously. He explains that emetine should not be employed in the treatment of carriers since it does not prevent the development of cysts in the majority of cases.

In the treatment of amebic hepatitis and amebic abscess of the liver the value of emetine is especially emphasized and it is stated that while the treatment of amebic abscess of the liver is largely a surgical problem, the earlier recognition and treatment of amebic hepatitis and beginning abscess formation is essentially a medical problem. He remarks that emetine may be said to be an absolute specific in the cure of amebic hepatitis and if this condition is recognized, a course of treatment with this drug is always successful in relieving the symptoms and preventing abscess formation and he notes if abscess formation has occurred, some authorities advocate the use of emetine alone, for the evidence is incontrovertible that this drug may cure even large abscesses of the liver. Puncture and aspiration of a liver abscess, Craig believes, should never be undertaken unless the patient has received injections of emetine for five or six days previously, especially if fever and marked enlargement of the liver are present. He adds that the administration of the emetine is followed by marked reduction in the congestion of the liver and greatly reduces the chances of hemorrhage during puncture of the organ. Usually liver puncture and aspiration of the contents of a liver abscess may be accomplished under careful novocain anesthesia but sometimes a general anesthetic is necessary. He explains that the technique of aspiration is simple and the reader is referred for this to the contributions by Ochsner and DeBakey (1943) for a description of this technique. He points out that the various methods of open operation have largely been abandoned for treatment with emetine followed by puncture and aspiration. *Emetine-Bismuth-Iodide:* The action of this drug, Craig writes, is undoubtedly like that of emetine alone but it is the concensus of opinion of those who have used it extensively that it is a more powerful amebacide than is emetine and that its curative value is much greater. It has been stated by some who are enthusiastic in its favor that it will cure practically 90% of amebic infections if properly administered but unfortunately the difficulty of its administration, owing to the unpleasant symptoms it almost invariably produces, prohibits its use as a routine treatment except in amebic dysentery and even here Craig believes it should only be used when the more specific drugs have failed. The greater amebacidal power of this preparation over emetine is probably due to its iodine content. Craig believes that the most efficient drugs we possess in the treatment of amebiasis have a high iodine content but even with the combination of emetine and iodine content in this drug it is certainly less efficient than the amebacidal drugs containing iodine alone. In this connection it is interesting to note that Manson-Bahr (Lancet, December 2, 1944) believes that combined treatment with emetine-bismuth-iodide and chiniofon now offers the best hope of a permanent cure but the details of administration must be observed.

Craig regards the iodine compounds as the most efficient and safest therapeutic agents for the elimination of the infection; chiniofon, diodoquin and vioform and entero-vioform are all discussed. *Chiniofon* (yatren, anayodin, or quinoxol), he believes from the literature available, to be a safe and efficient specific in the treatment of this condition. While it fails in certain infections, the records show that it is curative in the vast majority of amebic infections when properly administered and that serious toxic symptoms never occurred. He thinks that it should be preferred to any of the arsenicals that have been described because it is less toxic and can be used in mass treatment with safety and efficiency.

Diodoquin is also regarded as a most excellent nontoxic amebacide capable of eliminating amebic infection in most individuals.

Of the arsenicals *carbarsone* is regarded as the most powerful and the least toxic. Craig believes that treatment with this drug should be reserved for those infections which have proven incurable with the iodine compounds. Patients having liver or kidney disease obviously should not be treated with it.

There is a wealth of new material in the book but space prevents a longer review. The author is most generous in his consideration of the work of others and compares side by side their observations with his own. Where differences of opinion exist these are stated and Colonel Craig gives his personal point of view. This is fortunate since he is the most eminent authority upon the subject that we have.

[1] The term "amebacide" is used throughout the book. Dorland American Medical Dictionary, 1944, employs this spelling and states that "amebicide" is an incorrect spelling of "amebacide" but the authority is not given. Stedman's Medical Dictionary, 1939, gives "amebicide" and not "amebacide," a matter in which etymologists will be interested.

The book demonstrates in a way the decadence of scientific medicine in Germany, for while there are references to German investigations in earlier years, thirty-three German references being given, there is no mention of German work since 1927. The reason is clear. There has been no recent German work of outstanding value upon the subject.

RICHARD P. STRONG

Handbook of the Mosquitoes of North America. ROBERT MATHESON. Ithaca, N. Y.; Comstock Publishing Co., Inc., 1944. Pp. viii, 314. Illus.

The present war has brought to the fore the importance of malaria and other arthropod-borne diseases as military and home-front hazards. As a result there are probably more individuals directly concerned with the control of mosquitoes in this country and the world over than at any other time in history. It is particularly fortunate, therefore, that Doctor Matheson, Professor of Entomology at Cornell University, has produced a new, revised and enlarged edition of his authoritative "Handbook of the Mosquitoes of North America," which, since its original appearance in 1929, has been a valued guide to students of this branch of entomology. While the literature on North American mosquitoes is formidable in bulk and rich in detail, monographic works of continental scope have been few and long outstripped by rapid advances in knowledge. Here, in this slim volume of 314 pages, is condensed the fruits of many years of patient labor in the form of a convenient handbook that is essential equipment for mosquito-workers in the United States and Canada. It makes readily available the essence of our present knowledge of the taxonomy and biology of North American mosquitoes, much of which could otherwise be found, if at all, only in many separate publications and in older lengthy and cumbersome treatises now difficult to obtain.

The book is divided into two sections. Part I (86 pages) is a general survey of the structure and biology of mosquitoes, their relation to human welfare, their control, and methods of collecting and studying them—subjects of interest not only to the entomologist, but to epidemiologists, health officers, physicians, and laymen who will find here a clear and readable source of information. Part II (172 pages) is a systematic account of the North American species of mosquitoes—primarily of interest to the entomologist as a working tool.

The first part includes a detailed account of the morphology of the adult and larval stages which is fundamental to practical work of identification. The life cycle, breeding, feeding, and overwintering habits, knowledge of which is essential to practical control work, are briefly reviewed. The rôle of mosquitoes in the transmission of human malaria, yellow fever, dengue, filariasis, and virus encephalitides is concisely treated, with the malaria parasite cycle described and illustrated. The fundamental principles of control are outlined and the various types of control methods considered. Directions for collecting and preserving mosquitoes for study conclude this section of the book, which contains 41 good drawings and photographs to illustrate the textual material.

The second part provides means to identify systematically the larvae and adults of North American mosquitoes by appropriate keys to subgroups and species, and describes their group and specific characteristics. Descriptions of 133 species are given, 12 in the non-biting subfamily Chaoborinae and 121 in the Culicinae. The latter include all the blood-sucking species and are distributed among 11 genera (*Anopheles*, 13 species; *Aëdes*, 58; *Culex*, 19; *Culiseta*, 7; *Psorophora*, 10; *Mansonia*, 2; *Orthopedomyia*, 2; *Deinocerites*, 2; *Wyeomyia*, 3; *Uranotaenia*, 3; *Megarhinus*, 2). The Chaoborinae, which are poorly known, are treated briefly, but for each species of Culicinae there are included detailed descriptions of the female, the male genitalia, and the larva, and notes on distribution, breeding and feeding habits. Drawings of about 107 genitalia and 25 larvae are included in the 33 plates at the back of the book, which also illustrate typical breeding places. Mention is made of the known relation of the various anopheline species to the transmission of malaria; *Aëdes cantator* and *Culex tarsalis* are noted as vectors of equine encephalomyelitis and *Aëdes aegypti* of yellow fever and dengue.

A valuable addition to this new edition is the inclusion of over 200 classified bibliographic references, providing an excellent initial guide to the extensive literature on mosquitoes, not only of America but of other regions as well. The section on the Chaoborinae is also a welcome addition, as are the many new figures and the improved keys with their convenient page references. It is interesting to note the increase in number of species treated, from about 80 to 121 Culicinae, reflecting the increase in knowledge of this group in the fifteen years since the first edition appeared. (In 1901 L. O. Howard included only 25 species in the first general work on U. S. mosquitoes!) The space devoted to the anopheline mosquitoes has been doubled and five more species recognized within the States (*bradleyi*, *georgianus*, *freeborni*, and *franciscanus*, all tentatively raised from subspecific rank, and *albimanus* on our southern border). This edition adopts the generic names *Culiseta* for *Theobaldia* and *Mansonia* for *Taeniorhynchus*, in accord with current validated usage, and also indicates subgeneric groupings within the larger genera.

The necessarily brief treatment of mosquito-borne diseases and control includes only passing mention of the newer developments in these fields. Even in a brief summary, the interesting recent discoveries regarding the epidemiology and endemicity of yellow fever deserve more explicit mention than the author accords them. It is surprising that he does not use

the term "virus" to designate the causative agent of yellow fever ("the parasite . . . seems as yet unknown"), although he does for dengue and encephalitis. The publication of this edition followed hard upon the important advent of aerosols and DDT as promising new methods of attacking mosquitoes, and they are duly noted. In the chapter on techniques, no mention is made of light traps, chloral-gum mounting media, or dissecting methods for detecting malaria in mosquitoes, though these would well merit inclusion.

Fortunately, despite wartime restrictions, the book has been issued on the good quality paper that is necessary for durability and for clear printing of the many excellent illustrations. The format is neat and attractive, the typography uniformly good and effectively designed for ease of reading and reference. The text is remarkably free of printer's errors, and an adequate index insures the utility of the volume.

Professor Matheson is to be congraulated upon the successful completion of the herculean task of revision of his useful book. It is a mine of valuable information in the confines of a true "handbook." At this time it is more than ever evident that the practical aspects of mosquito-knowledge are literally a matter of life and death. Broadly conceived and sound academic studies such as this are fundamental to practical progress and provide part of the essential power in the complex machinery of combatting some of the more material ills that beset man.

ALBERT MILLER

Introduction to Parasitology. ASA C. CHANDLER. 7th Edition, John Wiley & Sons, Inc., New York. In 716 pages with 309 figures the author accurately presents the three branches of parasitology, namely; protozoology, helminthology, and entomology and the spirochetes.

The author has a very smooth and attractive language, which retains the interest of the reader. The life cycles and morphology of the parasites and the epidemiology and prevention of the diseases caused by them are discussed with great accuracy, but for the clinical phases of the diseases the reader should consult text books on clinical medicine.

Rat-bite fever should not be included with the spirochetal infections because *Spirillum minus* is a bacterium and not a spirochete. According to the author the organism shows affinities with the spirochetes in being non-culturable, in the type of disease it causes, and its susceptibility to treatment with arsphenamine. The facts are that the organism can be cultured, the disease may resemble bacterial or protozoal infections and that other diseases respond to arsenic. On the other hand, no mention is made of bejel and irkintja. On page 49 it is stated that louse borne relapsing fever is present in Mexico and Peru. At the present time there is no louse borne relapsing fever in the Western Hemisphere. There are very few mistakes and errors except for misspelled words and typographical errors. For example, on page 131 in the crithidia, the flagellum arises from the kinetoplast which has shifted forward in front of the nucleus rather than behind the nucleus.

This book is one of the standard text books on parasitology and it is recommended that the student of parasitology and the physician who is practicing tropical medicine should consult it frequently.

HARRY SENEKJIE

ON DIFFICULTIES ARISING IN THE EXPERIMENTAL PROPAGATION OF FALCIPARUM MALARIA[1]

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The continued propagation of falciparum malaria, either wholly naturally, or through the resumption of natural passage after artificial propagation, has proved in our experience more difficult than that of vivax malaria. Breaks from inoculation failures have occurred in the continuity of anopheline-human passages of all of the strains of *Plasmodium falciparum* which we have attempted to propagate routinely, while gametocyte production has become precarious with strains propagated artificially. The present paper is an attempt to analyze the conditions under which these difficulties arose, in order to elucidate, if possible, some of the responsible factors.

Plasmodium falciparum has been employed in our service at the Florida State Hospital since June 1932, largely for the inoculation of colored patients. During this period of approximately thirteen years, we have employed several strains, for the most part of indigenous origin, although we have also had available a few exotic strains presumably from the American tropics. To the former category belong those designated as Coker, Long, Costa, Spears, Thomas, Perkins, and Bynum; to the latter, the Cuban, Mexican, Panamanian, and Trinidadian. The Coker, Long, and Costa strains were successively used for routine therapeutic inoculations over extended periods of time. The natural inoculations effected with these strains, comprising 108 inoculations of white patients and 278 inoculations of colored patients and totaling 386 inoculations and reinoculations, are epitomized in table 1. Of these, takes were secured on primary inoculation in 71.2 per cent of white patients and 67.5 per cent

TABLE 1

Takes and Failures Following Natural Inoculations with P. falciparum

STRAIN	PRIMARY				SECOND (REINOCULATION)				THIRD (REINOCULATION)				TOTAL				
	White		Colored		White		Colored		White		Colored		White		Colored		
	Takes	Fail.	Takes	Fail.	Takes	Fail.	Takes	Fail.	Takes	Fail.	Takes	Fail.	Takes	Fail.	Takes	Fail.	All
Spears			3												3		3
Coker	3	2	30	23		1	8	7		1	3	1	3	4	41	31	79
Long	31	15	89	30		2	6	1					31	17	95	31	174
Costa	33	6	29	13		2	2						33	8	31	13	85
Thomas			4	2											4	2	6
Perkins	1	2	5	6		1							1	3	5	6	15
Cuban			2	2			1								3	2	5
Mexican	1	1	4	1			2						1	1	6	1	9
Panamanian			1	1											1	1	2
Bynum	2												2				2
Trinidad	1	3		2									1	3		2	6
Total	72	29	167	80		6	19	8		1	3	1	72	36	189	89	386
White																	108
Colored																	278

[1]The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of The Rockefeller Foundation, in cooperation with the Florida State Board of Health and The Florida State Hospital.

TABLE 2

Experience with Various Mosquito Lots Employed in Consecutive Human-Anopheline Passages of Various Strains of P. falciparum

ORIGINALLY RECOVERED FROM	STRAIN	RESULT	CONSECUTIVE PROPAGATION																										TOTALS		
			1		2		3		4		5		6		7		8		9		10		11		12		13				
			L*	R†	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	F‡ L	T§ L	T R
Autochthonous infection	Thomas	Takes	1																											1	
		Failures																													
	Spears	Takes	1																											1	
		Failures																													
	Coker	Takes		1																											1
		Failures			1																								1		
	Perkins	Takes		1		1																									2
		Failures					1																						1		
Blood inoculated patient	Coker	Takes	1	3	1	1		1		1		1	1	1		1														3	9
		Failures	5		2				1		1						1												10		
	Long	Takes	1	5	1	4		2		3		3	3	2		1	1	1		1	1	1		1	1	1	2			10	25
		Failures	3		1		2								1														7		
	Cuban	Takes	1																											1	
		Failures	1																										1		
	Mexican	Takes	1	2	2																									3	2
		Failures																													
	Panamanian	Takes	1																											1	
		Failures																													
	Costa	Takes	4	4	2	3	1	2	1	1		1		1		1		1	1											9	14
		Failures	2		1						2				1														6		

	Trinidad	Takes	1																											1	
		Failures	1																										1		
	Bynum	Takes	1																											1	
		Failures																													
Totals		Takes	13	16	6	9	1	5	1	5		5	4	4		3	1	2	1	1	1	1		1	1	1	2			31	53
		Failures	12		5		3		1		3				2		1												27		

* L = strain not subsequently recovered.
† R = Strain recovered in anophelines.
‡ F = Failure or all propagation unsuccessful.
§ T = Take or propagation successful.

of colored patients. The differences are not significant. The inoculations discussed were effected with 111 different lots of falciparum-infected *Anopheles quadrimaculatus*.

Inoculations were made with varying numbers of mosquitoes selected from lots at different intervals after the completion of the extrinsic incubation period. Reference in following tables to *age of sporozoites in days* is to days elapsing from the completion of the extrinsic incubation period.

The numbers of mosquitoes applied varied with the incidence of infection in the lot as determined from the observation of stomach infections in specimens dissected during the extrinsic incubation period, but always with the idea of effecting the inoculation with an excess of sporozoites. As a general rule the mosquitoes were employed but once for inoculation, and within 24 hours their salivary glands were dissected and examined. In most instances, all the inoculations effected by different mosquitoes selected from a lot were made simultaneously, but in a small number of cases, withdrawals were made for consecutive inoculations over periods as long as three weeks. These mosquitoes were, however, used but once. As a rule, later selections of mosquitoes for inoculations were not made from a lot when the prior application of representative specimens had not resulted in takes after the lapse of the characteristic mean prepatent period.

TABLE 3

Circumstances Attending Fifteen Breaks in Natural Passage

STRAIN	PASSAGE SERIES	PENULTIMATE PASSAGE									UNSUCCESSFUL ULTIMATE PASSAGE								
		Mosquitoes employed					Patients				Mosquitoes employed					Patients			
		per cent infec. lot	Infected				Takes		Failed		per cent infec. lot	Infected				Takes		Failed	
			Number		Age spor. in days							Number		Age spor. in days					
			Min.	Max.	Min.	Max.	W.	C.	W.	C.		Min.	Max.	Min.	Max.	W.	C.	W.	C.
Coker	a	29.8	1	1	1	1	0	1	0	0	25.0	1	1	4	13	0	0	0	4
	b	71.0	1	2	7	20	1	6	0	0	24.7	2	2	14	16	0	0	0	3
	c	52.5	1	5	8	12	1	2	0	0	87.5	4	7	32	38	0	0	0	3
	d	75.2	4	9	5	32	0	4	0	2	54.5	8	8	30	30	0	0	0	1
	e	64.4	3	6	6	8	0	7	0	0	88.2	4	13	7	32	0	0	2	6
	f	82.4	5	6	18	23	0	2	0	1	50.7	4	6	16	20	0	0	0	2
Long	a	86.6	4	9	16	28	0	3	1	0	79.2	5	12	14	17	0	0	0	4
	b	31.5	1	3	5	5	0	1	0	4	20.0	5	6	8	11	0	0	2	0
	c	35.2	3	3	3	3	0	1	0	0	26.6	2	2	5	5	0	0	0	1
	d	90.0	4	8	13	19	0	3	3	0	87.0	4	6	3	3	0	0	0	5
Perkins	a	46.9	4	15	11	21	0	4	0	0	71.2	3	8	14	29	0	0	2	5
Costa	a	64.4	5	5	4	7	0	4	0	0	65.2	2	2	9	9	0	0	1	0
	b	75.6	7	7	3	5	3	0	1	0	47.0	4	5	9	9	0	0	2	0
	c	66.6	4	8	6	6	0	1	0	2	19.4	2	5	7	8	0	0	2	0
	d										23.8	5	5	7	7	0	0	1	0

Based on the results secured from their application, the different mosquito lots may be conveniently grouped as follows:

a) No takes resulting from 27 lots employed in 67 inoculations.

b) Application resulting in a variable proportion of takes and failures—34 lots producing 91 takes and 58 failures in 149 inoculations.

c) Takes resulting from all applications of 50 lots employed in 170 inoculations.

The strains represented by these 111 mosquito lots were originally recovered either from patients with autochthonous infections, or from patients artifically subinoculated from the original autochthonously infected patient. The extent to which

we were able to maintain them by subsequent consecutive anopheline-human passages, is shown in table 2. It will be seen that 13 consecutive human-anopheline passages were effected with the Long strain, and runs of 7 and 9 were secured with the Coker and Costa strains.

shown in table 3. It should be stated that all of the patients in the ultimate series of propagation before the 15 breaks (12 white, 34 colored), were bitten by varying numbers (from 1 to 13) of demonstrably infected anophelines, yet none became infected. The numbers of infected mosquitoes

TABLE 4

Numbers of Infected Mosquitoes Used in the Penultimate and Ultimate Series of Inoculations before the Fifteen Breaks, Compared with the Age of Their Sporozoites

AGE SPOROZOITES	SERIES	NUMBERS OF INFECTED MOSQUITOES							
		1–5		6–10		11+		Total	
		Inoc.	Takes	Inoc.	Takes	Inoc.	Takes	Inoc.	Takes
days									
1–5	Penultimate	9	5	5	4			14	9
	Ultimate	5	0	2	0			7	0
6–10	Penultimate	14	14	4	2			18	16
	Ultimate	10	0	1	0			11	0
11–20	Penultimate	10	8	7	6	2	2	19	16
	Ultimate	9	0	4	0	2	0	15	0
21+	Penultimate	2	0	4	2	1	1	7	3
	Ultimate	7	0	5	0	1	0	13	0
Total	Penultimate	35	27	20	14	3	3	58	44
	Ultimate	31	0	12	0	3	0	46	0

TABLE 5

Numbers of Infected Mosquitoes Employed from Twenty-seven Lots Giving Sixty-seven Unsuccessful Inoculations, Compared with Age of Their Sporozoites

AGE SPOROZOITES	NUMBERS OF INFECTED MOSQUITOES							
	1–5		6–10		11+		Total	
	Inoc.	Take	Inoc.	Take	Inoc.	Take	Inoc.	Take
days								
1–5	5	0	2	0			7	0
6–10	14	0	4	0			18	0
11–20	11	0	8	0	2	0	21	0
21+	14	0	6	0	1	0	21	0
Total.....	44	0	20	0	3	0	67	0

As noted, there are 27 breaks in continuity of passage due to inoculation failures after the application of demonstrably infected mosquitoes. The circumstances attending 15 of these breaks, when the unsuccessful inoculation had been preceded by at least one successful passage, is

employed in these inoculations, and the age of their sporozoites, are compared in table 4, further distinguishing between the unsuccessful ultimate passages, and the corresponding penultimate passages with varying proportions of takes. The inoculations effected in either series did not sig-

nificantly differ in the number of infected mosquitoes applied or in the age of their sporozoites. Yet 76.0 per cent of takes were secured in the penultimate series, and none resulted from the inoculations in the ultimate series. It is not evident that the universal terminal failures can be generally ascribed either to a deficiency of infected mosquitoes or to excessive age of their sporozoites.

lots (distinguishing the 15 more specifically mentioned), is shown in table 6.

While the incidence of infection was less than 50 per cent in 12 of the 27 lots, we would, nevertheless, regard the remainder as of good quality.

For purposes of maximum contrast, we may next turn to a consideration of the 170 takes resulting from the employment of the 50 lots giving

TABLE 6

Percentage Incidence of Infection in All Mosquitoes Included in the Twenty-seven Unsuccessful Lots, Distinguishing the Fifteen More Specifically Mentioned

LOT GROUP	PER CENT INFECTION IN UNSUCCESSFUL LOTS										
	−10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90	91–100	Total
Causing break	—	2	4	—	1	2	1	2	3	—	15
Other lots	1	—	1	2	1	—	1	3	1	2	12
Total	1	2	5	2	2	2	2	5	4	2	27

TABLE 7

Numbers of Mosquitoes in Fifty Lots Giving Universal Takes, Compared with Age of Their Sporozoites

AGE SPOROZOITES	NUMBERS OF INFECTED MOSQUITOES							
	1–5		6–10		11+		Total	
	Inoc.	Take	Inoc.	Take	Inoc.	Take	Inoc.	Take
days								
1–5	9	9	13	13	0		22	22
6–10	49	49	32	32	10	10	91	91
11–20	26	26	16	16	9	9	51	51
21+	2	2	3	3	1	1	6	6
Total	86	86	64	64	20	20	170	170

The number of infected mosquitoes employed from the 27 lots giving the 67 unsuccessful inoculations are compared with the age of their sporozoites, in table 5. Data from table 4 are included. It is again seen that these failures have not borne any consistent relation to age and dosage of sporozoites.

Furthermore it is unlikely that many, if any, of these failures can be attributed to a pre-existing homologous immunity, as 31 of all colored patients in whom inoculations failed were later naturally inoculated with the homologous strain, 22 takes resulting, and 13 were later artificially inoculated, with 13 takes.

The percentage incidence of infection in all of the mosquitoes included in the 27 unsuccessful

universal takes. The number of mosquitoes and the age of their sporozoites are shown in table 7. The sporozoites of the mosquitoes considered in table 7 were significantly younger at the time of inoculation than those of the mosquitoes listed in table 5, as shown by a Chi-square of 40.33, $P < 0.01$. This indicates that difference in the age of sporozoites has been a significant factor in producing the results described. The incidence of infection in the successful lots is shown in table 8. A Chi-square comparison of the distribution in tables 6 and 8 indicates that successes and failures from inoculations were similarly grouped with respect to the qualitative level of infection in the lots at more or less than 50 per cent.

The 149 inoculations effected with the 34 mos-

quito lots producing both takes and failures, are considered in table 9 from the standpoint of number of infected mosquitoes and the age of their sporozoites.

A Chi-square test indicates that the takes and failures from the application of these mosquitoes were significantly related to the age of their sporozoites. A Chi-square of 13.24, $P < 0.01$, was secured.

TABLE 8

Infection in Successful Mosquito Lots

PER CENT INFECTION IN SUCCESSFUL LOTS										
–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90	91–100	Total
1		6	4	4	5	13	7	3	7	50

monstrably parasitized mosquitoes infected at these lower densities. This of course is to be expected in view of the dissection criterion employed.

Of the 111 mosquito lots considered, 89 were infected during the first gametocyte wave. These comprise 80 per cent of the lots which were wholly effective, 57 per cent of those which gave partial takes, and 89 per cent of those which were failures. The mean number of days elapsing from the first appearance of gametocytes to the application of mosquitoes for infection were, in the order followed above, 14.9, 14.2, and 11.2, slight differences, which in view of the scatter, are not regarded as significant.

The induced falciparum infections frequently require some degree of therapeutic interference

TABLE 9

Inoculations Effected with Thirty-four Lots of Mosquitoes Producing Both Takes and Failures, Showing Numbers of Infected Mosquitoes and Age of Sporozoites

AGE SPOROZOITES	NUMBERS OF INFECTED MOSQUITOES							
	1–5		6–10		11+		Total	
	Inoc.	Takes	Inoc.	Takes	Inoc.	Takes	Inoc.	Takes
days								
1–5	16	5	6	5	4	2	26	12
6–10	41	30	9	3	9	7	59	40
11–20	14	9	30	24	5	2	49	35
21+	4	0	11	4	0		15	4
Total.....	75	44	56	36	18	11	149	91

The number of natural inoculations effected with exotic strains was small (22). In comparison with indigenous strains the proportion of takes is somewhat lower, but in view of the small numbers of inoculations involved, the data are not regarded as significant.

The infectiousness of the patients from whom the mosquitoes, included in these groups of 27 and 50 lots, derived their infection, is compared in table 10. Although in both series of lots (A: giving no takes; B: giving all takes) a trend is apparent for the incidence of infection in the mosquito lots to increase directly with the gametocyte density prevailing in the infecting patient, roughly one-third of the lots in each series was nevertheless infected at densities not exceeding 500. gametocytes per c.mm., and many takes actually resulted from the application of de-

shortly after their onset, to control the exuberance of their evolution. We commonly employ quinine for this purpose. It is reasonable to assume that all traces of quinine will have disappeared from the body by the end of 10 days after the administration of the last dose, although it is conceivable that some of the gametocytes still in circulation after that interval actually came in contact with the last traces of the drug during their developmental period.

In table 11 there is presented a summarization of the extent to which quinine had been administered to the patients on whom the various mosquito lots were infected, further indicating whether the last dose administered was given within 10 days or less of their application, or a longer interval had elapsed. This table further presents the inoculation results secured from the

application of the mosquitoes from these lots considered from the standpoint of the quinine administered.

lots were infected, does not reveal a significant relationship. A comparison of takes and failures among the 239 patients whose mosquitoes had

TABLE 10

Correlation of Gametocyte Density with Resultant Infection in Mosquitoes

TOTAL GAMETOCYTES PER C.MM.	PER CENT INFECTION IN LOT																				TOTAL	
	−10		11-20		21-30		31-40		41-50		51-60		61-70		71-80		81-90		91-100		Giving no takes "A"	Giving all takes "B"
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B		
None																					0	0
Less than 100																	1				1	0
101–500			2		4	3	1	2		2	1	2		5		1	1	1			9	16
501–1000					1	1	1					1		4	1				1	5	4	11
1001–2000						1		1		2		1	1	1	2			1		2	3	9
2001–3000	1													2	1	3	1		1	1	4	6
3001+		1							1			1		1	1	3					2	6
Not enumerated						1		1	1		1		1				1				4	2
Total:																						
A	1		2		5		2		2		2		2		5		4		2		27	
B		1				6		4		4		5		13		7		2		8		50

TABLE 11

Amount and Time of Quinine Administration to Infector Prior to the Application of Mosquitoes

RESULT OF APPLICATION OF LOTS	TOTAL		QUININE ADMINISTRATION TO MOSQUITO LOT INFECTOR PRIOR TO APPLICATION OF MOSQUITOES				
				Ten grains or less within		Eleven grains or more within	
	Lots	Patients inoc.	None	a) More, or than the ten days prior to mosquito feeding	b) Less than the ten days prior to mosquito feeding	c) More, or than the ten days prior to mosquito feeding	d) Less than the ten days prior to mosquito feeding
All takes	48 (+2*)		17	5	5	13	8
		166 (+4*)	51	15	13	58	29
Partial takes	32 (+2*)		16	5	2	4	5
takes		84 (+7*)	44	14	3	9	14
failures		55 (+3*)	27	4	7	6	11
All failures	27		5	6	3	4	9
		67	11	18	8	10	20
All lots	107 (+4*)		38	16	10	21	22
All patients							
takes		250 (+11*)	95	29	16	67	43
failures		122 (+3*)	38	22	15	16	31
total		372 (+14*)	133	.51	31	83	74

* Lacking data *re* quinine.

A Chi-square analysis of all takes and failures on inoculation, compared with the quinine experience of the patients on whom the respective mosquito

been infected on patients receiving quinine, considered as to whether the last quinine had been administered either more than 10 days (column

a,c) or less than 10 days (column b,d) from the date of their infection, gives a Chi-square of 6.17, with a probability value of $0.05>P>0.01$. A further consideration of takes and failures in the 157 patients inoculated by mosquitoes infected on patients who had received 11 grains or more of quinine within either more (column c) or less (column d) than 10 days of the infection of these mosquitoes, gives a Chi-square of 9.425 with a probability value of $P<0.01$. These observations indicate that the administration of quinine to the patients on whom the inoculating mosquitoes were infected, has been prejudicial to the vitality of the parasites. While it is well known that mosquitoes can be infected by the gametocytes in patients with falciparum malaria who are receiving quinine, it has been assumed that the parasites resulting from the completion of the subsequent sporogonous cycle were unimpaired. These data indicate that even though the gametocytes were able to infect the mosquitoes and the sporogonous cycle was completed therein, the resulting sporozoites are frequently deleteriously affected and become unable to infect the patients to whom they are later applied.

The age of the sporozoites in the infecting mosquitoes is compared in table 12 with the results from their application, further distinguishing as to whether or not the mosquitoes had been infected on patients receiving quinine.

These results do not indicate that the effect of quinine has accelerated the deleterious effect of age on the sporozoites.

The mean number of infected mosquitoes and the mean age of their gland infection in all inoculations are classified according to the results following their employment in table 13:

The data presented in tables 9, 12, and 13 corroborate the fairly rapid deterioration of falciparum sporozoites with age, to which we called attention some years ago (Boyd, Stratman-Thomas, and Kitchen, 1936). This suggests the existence of a rather high falciparum sporozoite threshold. Such might be consequent to a diminished vitality of the sporozoites resulting from the administration of quinine to the patient from whom their progenitor gametocytes were derived.

TABLE 12

Age of Sporozoites in Infecting Mosquitoes Compared with Results from Their Application, with Indication as to Whether Mosquito Infectors Have Received Quinine

AGE OF SPOROZOITES	NO QUININE GIVEN TO MOSQUITO-INFECTING PATIENTS				QUININE GIVEN TO MOSQUITO-INFECTING* PATIENTS			
	Failures		Takes		Failures		Takes	
	All	Partial		All	All	Partial		All
days								
1–5		7	5	5	7	5	5	15
6–10	2	7	23	25	16	11	14	66
11–20	5	9	16	19	16	5	17	30
21+	4	4		2	17	7	4	4
Total	11	27	44	51	56	28	40	115
Mean age	17.6	11.7	10.2	11.0	14.7	12.7	12.3	9.7
SE	±1.8	±1.1	±0.9	±0.8	±0.9	±1.3	±1.1	±0.6

* Less 14 patients for whom quinine data are lacking.

TABLE 13

Mean Number of Infected Mosquitoes and Mean Age of Their Gland Infection, All Inoculations. Classified According to Results of Their Application

RESULTS	NUMBER OF LOTS	PATIENTS INOCULATED	MEAN NUMBER INFECTED ANOPHELINES	MEAN AGE SPOROZOITES
				days
All takes	50	170	6.2	10.0
Partial takes	34	91	6.4	10.7
Partial failures	34	58	6.1	11.7
All failures	27	67	5.0	14.7

Artificial propagation of strains has been performed under the following circumstances:

a) An artificial subinoculation to secure an infectious patient when the original autochthonous patient resided at a distance, currently had a low gametocyte density, or a latent infection.

b) When breaks in the continuity of natural inoculation passage necessitated an artificial subinoculation to preserve the strain, in the hope that the recipient would produce sufficient gametocytes for the infection of mosquitoes.

c) For the preservation of strains which were not being used for routine inoculations.

TABLE 14

Passages Effected during Artificial Propagation of Strains

STRAIN	NUMBER OF ARTIFICIAL PASSAGES BEFORE INFECTION OF MOSQUITOES							
	1	2	3	4	5	6	7	8
Spears	1							
Coker	2	2						
Long	3	2	2					1
Costa	2	3	1	1	1			
Thomas	1							
Perkins						1		
Cuban		1	1					
Mexican			1	2		1		
*Panamanian					1			
Bynum		1						1
*Trinidad	1							
Harden		1					1	
Total	10	10	5	3	2	2	1	2

* Mosquito lots were not infected after the later passage of these strains.

The series of artificial passages effected during the propagation of strains are shown in table 14.

In many instances anophelines were successfully infected from a single artificially inoculated patient, in others a low level of infectiousness in the successive patients artificially inoculated prevented satisfactory recovery of the strain in mosquitoes. The present examination is limited to a consideration of low infectiousness in those consecutive artificial propagations consisting of 5 or more passages, and involves the Long, Costa, Perkins, Mexican, Panamanian (2 series), Bynum, Trinidad, and Harden strains. Except in one instance, all inoculations which were intravenous, resulted in takes. Termination of a series of artificial passages occurred either because (a) mosquitoes were finally successfully infected, or (b) the strain was deliberately discontinued. Strains were discontinued either when congestion of wards or temporary insufficiency of patients prevented their further propagation, or when it appeared that gametocyte production had become so unreliable that later recovery in mosquitoes appeared impracticable. There are considered 31 inoculations of white patients and 101 inoculations of colored patients.

The maximum gametocyte densities observed in these patients are classified in table 15, in which

TABLE 15

Maximum Gametocyte Densities in Lineal and Collateral Passages, 132 Inoculations

STRAIN	MAXIMUM GAMETOCYTES PER C.MM.							
	None		—100		101–1000		1001+	
	Lin.	Col.	Lin.	Col.	Lin.	Col.	Lin.	Col.
Long			1		6		1	3
Costa					4		2	3
Perkins	4	1	1			3	1	1
Mexican							7	2
Panamanian (1)				2	4	4	1	1
Panamanian (2)	3	2	11	3	6	1	1	
Bynum		2	1	1	3	2	4	
Trinidad	5	6	8	3	3		3	1
Harden	1			1	1	2	5	1
Total lineal	13		22		27		25	
Total collateral		11		10		12		12

patients from whom successive propagative inoculations were made are grouped as "lineal", and those from whom no subinoculations were made are grouped as "collateral."

In 24 patients no gametocytes were observed, while in 32 others the maximum density observed did not exceed 100 per c.mm. We regard a falciparum gametocyte density not exceeding 100 per c.mm. as inadequate to effect reliable infection of mosquitoes. The passages in which the gametocytes were low or absent are shown in table 16.

TABLE 16

Fifty-six Passages in Which Gametocytes Were Low in Density or Absent

STRAIN	GAMETOCYTES	PASSAGE																						TOTAL	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22–24	0	–100
Long	0																								
	–100							1																	1
Costa	0																								
	–100																								
Perkins	0			1	1	2	1																	5	
	–100		1																						1
Mexican	0																								
	–100																								
Panamanian (1)	0																								
	–100			2																					2
Panamanian (2)	0						1							1		1			1	1				5	
	–100	1						1	2	1	1	1	1	1	1			1	1		1	1			14
Bynum	0			1		1																		2	
	–100	1		1																					2
Trinidad	0							1	2			1		1	1	4	1	1*	1*	1*	1*	1*	3*	11(+8*)	
	–100		1			1				1	1	1	1		1	2	2								11
Harden	0							1																1	
	–100				1																				1

* Not included in subsequent analyses.

The experience with the Perkins, Panamanian, and Trinidad strains, indicates that when gametocyte production became poor or altogether lacking, this characteristic was retained in many subsequent passages.

Some of the clinical and parasitological features of these infections are presented in table 17.

It is evident that differences are associated with the racial status and immunological background of the patients. The racial distinctions are conserved in the subsequent analyses.

The question of possible immunity may next be considered. It may be said that the classification in the "known" group is exact, as it refers to previous inoculations on our service with homologous or heterologous strains of this parasite. On the other hand, the complete susceptibility of the "unknown" group cannot be inferred. In table 18 the gametocyte density is considered according to race of patients and history of previous falciparum malaria.

A Chi-square test, computed from a fourfold table separating the colored patients on the basis of their past experience with falciparum malaria, and as to whether their maximum gametogeny exceeded or fell below 100 gametocytes per c.mm. gives a Chi-square of 6.15, which has a probability value of $0.02 > P > 0.01$. This does not indicate

that an acquired immunity has adversely affected gametogeny on subsequent heterologous reinoculations, but rather suggests that it actually may be enhanced thereby. Thus an acquired immunity on the part of colored patients at least is not a factor in the production of the deficiency considered. This observation would appear to have important epidemiological implications.

TABLE 17

Clinical and Parasitical Features of Infections in Which Gametocyte Production was Low or Absent

RACE	PREVIOUS INOCULATION WITH P. FALCIPARUM	NUMBER PATIENTS	NUMBER WITHOUT GAMETOCYTES	MEAN					QUININE GIVEN TO
				Days duration illness	Days with fever	Max. density: Trophozoites in thousands	Max. density: Gametocytes in thousands	Grains quinine administered	
						Per c.mm.			
White	Known	8	1	7.5	6.25	46.1	.79	12.3	6
	Unknown	23	2	38.5	21.4	106.4	2.81	31.1	23
Colored	Known	25	3	13.4	7.4	46.4	1.39	16.6	9
	Unknown	76	18	23.0	12.0	66.5	1.40	20.6	61
Total		132	24						99

The usual therapeutic intervention was frequently required in the management of these attacks. The amount of quinine administered is shown below in table 19.

From a fourfold table prepared by segregating the colored patients who had not receive quinine from those who had, further subdividing on the basis of whether maximum gametogeny was more or less than 100 per c.mm. a Chi-square of 2.085 is secured, which is not significant. It does not appear that the quinine administered had adversely affected gametogeny in either white or colored patients.

The extent to which deficient gametogeny in a donor is reflected in the patient inoculated with his parasites, is shown in table 20.

A Chi-square computation from a fourfold table in which colored donors are separated on the basis of 100 gametocytes per c.mm. more or less, and compared with similar maximal densities in their respective colored recipients, gives a Chi-square of 50.29, with Yates' correction, which has a value of $P<0.001$. This indicates that when gametogeny has been low in the donor, it is likely to be poor in the subinoculated recipients.

The three strains, Perkins, Panamanian (2nd series), and Trinidadian, in which gametogeny appears to have failed, were entirely propagated in colored patients (table 16). In the Panamanian strain gametogeny was irregular, being suppressed in the sixth, fifteenth, and nineteenth passages of lineal propagation, and low in all others subsequent to the fifth. It became low on the second passage of the Perkins strain, and failed altogether in the

TABLE 18

Gametocyte Density in 132 Patients, According to Race of Patient and History of Previous Falciparum Malaria

RACE	PREVIOUS FALCIPARUM MALARIA	MAXIMUM DENSITY OF GAMETOCYTES IN THOUSANDS PER C.MM.				TOTAL
		None	−0.1	0.1–0.99	1.0+	
White	Known	1	2	4	1	8
	Unknown	2	1	6	14	23
Colored	Known	3	4	11	7	25
	Unknown	18	25	17	16	76
Total		24	32	38	38	132

third and subsequent passages. In the Trinidadian it failed in some patients in the seventh, eighth, and eleventh, and from the thirteenth to the seventeenth and subsequent passages. When resumed in others between the ninth and sixteenth passages it was at a level below 100 per c.mm. On the other hand propagation of the Long, Panamanian (first series), and Mexican strains in colored patients did not result in failure of this function.

It is not evident from the limited data that

subinoculation of falciparum strains from a white patient who has a low density of gametocytes will perpetuate this condition in the recipients

TABLE 19

Amount of Quinine Administered in Therapeutic Intervention

RACE	TOTAL QUININE IN GRAINS ADMINISTERED	GAMETOCYTES IN THOUSANDS PER C.MM.				
		None	−0.1	0.1–0.99	1.0+	Total
White	None	0	0	2	0	2
	−10	1	3	3	5	12
	11–60	1	0	0	8	9
	61+	1	0	5	2	8
Total.....		3	3	10	15	31
Colored	None	6	6	10	9	31
	−10	6	10	7	5	28
	11–60	8	12	8	8	36
	61+	1	1	3	1	6
Total.....		21	29	28	23	101

or complete failure of gametogeny has developed either gradually (Panamanian, Trinidad) or suddenly (Perkins) in different strains. Deterioration of gametogeny has become apparent after passages by artificial inoculation varying from two to six in number. This suggests a deterioration in the reproductive capacity of the parasite strain involved.

Considering the 33 patients in the Perkins, Trinidadian, and Panamanian (second) series in whom gametogeny failed, the mean interval from the appearance of trophozoites in the donor until the subinoculation was 41.06 days, and in 29 other patients in whom gametogeny did not fail, the mean interval is 50.0 days. On reclassifying the above-mentioned 62 inoculations on the basis of whether the subinoculum was removed during clinical activity or latency in the donor, the subinoculation was made when 19 were clinically active and 43 latent. In the former the mean interval from the first appearance of trophozoites in the donor to the removal of the inoculum was 26.05 days, in the latter 53.72 days. Gametogeny failed in 9, or 47.4 per cent, of inoculations made

TABLE 20

Extent to Which Deficient Gametogeny in a Donor is Reflected in Patient Inoculated with his Parasites

Maximum density of gametocytes in thousands per c.mm.

IN DONOR		IN RECIPIENT									
		None		−0.1		0.1–0.99		1.0+		Total	
		W	C	W	C	W	C	W	C	W	C
None	W										
	C		7		5		1				13
−0.1	W					1				1	
	C		11		16		1		1		29
0.1–.99	W	1		1		3		5		10	
	C		3		5		19		7		34
1.0+	W	2		2		5		8		17	
	C				3		7		14		24
Total.....	W	3		3		9 + 1		13 + 2		*28 + 3	
	C		21		29		28		22 + 1		100 + 1

* Plus three white and one colored from undetermined donor with more than 0.1 thousand gametocytes.

inoculated. On the other hand, low gametogeny may be perpetuated in colored patients when subinoculated from other colored patients with a deficient gametogeny. In our series the weakening from clinically active donors, and in 24, or 55.8 per cent, of inoculations made from latent donors. Deterioration first became apparent in subinoculations made from clinically latent donors, 117

days (Perkins), 47 days (Panamanian), and 95 days (Trinidad) after the first detection of trophozoites in their infection. It does not appear that an excessive interval from the first detection of parasites in the donor to the removal of the inoculum has contributed to the failure of gametogeny, although the possibility that clinical latency in the donor was detrimental cannot be eliminated.

In an effort to resuscitate the lost property of gametogeny in the Trinidad strain after complete failure of gametogeny from the sixteenth to the eighteenth passages, a series of six further transfers were made, removing the inoculum from the consecutive donors from the second to the seventh day after the first appearance of trophozoites, and during maximum clinical activity. Although the the infections thereby induced attained much higher maximal trophozoite densities, the severity of the infections were not otherwise enhanced. No gametocytes were detected at any time in any of these patients. Thus the Trinidad strain in the last nine passages has been completely agametocytogenic.

SUMMARY AND CONCLUSIONS

Attention is directed to the difficulties we have experienced in the propagation by either natural or artificial means of various strains of *Plasmodium falciparum*. Difficulties arising during the course of natural propagation are manifested by the failure of all or a part of the inoculations to take, even though performed by demonstrably infected mosquitoes. In view of the dissection criteria employed to check inoculations, it would not appear that the gametocyte level in the patient from whom the mosquitoes were infected has been a factor. Neither would it appear that refractoriness of the patients inoculated has been a frequent obstacle, since takes have commonly resulted on the reinoculation of such patients. An increasing proportion of failures is associated with increasing age of the sporozoites present in the mosquitoes, a characteristic of which we were already aware. This deterioration is more rapid than is the case with vivax sporozoites. However, since many failures occurred when fresh or relatively fresh sporozoites were used, this cannot be the sole factor involved. A further factor elicited results from the comparatively recent ingestion of quinine by the patient on whom the mosquitoes were infected. It has not prevented completion of the sporogonous cycle, but nevertheless it has impaired the vitality of the resulting sporozoites.

When certain of these strains were propagated artificially, gametogeny deteriorated suddenly or gradually. This change has been observed in strains propagated in colored patients. Its occurrence does not appear to be consequent on a heterologous immunity in the patients, since it has more commonly been observed in those presumed to be susceptible. This change does not appear related either to the administration of quinine to the patient or to its amount. When once initiated it appears to persist throughout subsequent passages, and we have not been able to overcome this deterioration.

Failure of gametogeny in strains of *Plasmodium vivax* which have been artificially propagated for extended periods in the application of malaria therapy has been reported. We succeeded in acquiring two strains with such a reputed characteristic, and, while these were being propagated on our service abundant gametocytes were detected, mosquitoes were infected, and infection was transmitted therewith. Our experience with this species of parasite leads us to suspect that reports of failure of gametogeny indicates lack of familiarity with the morphology of the vivax gametocytes on the part of the microscopists concerned. On the other hand, the morphology of the gametocytes of *Plasmodium falciparum* is sufficiently distinctive to render credible any reports of their presence or absence.

REFERENCE

Boyd, Mark F., Stratman-Thomas, W. K., and Kitchen, S. F.: On the Duration of Infectiousness in Anophelines Harboring *Plasmodium falciparum*. Am. Jour. Trop. Med., **16**: 157–158, 1936.

TABLE 1

Results of Experiment A

PAIR	PATIENT'S NUMBER	RÔLE	QUINACRINE HYDROCHLORIDE				INOCULATION			RESULTS								
			Day of initial dose	Relation of initial dose to inoculation	Regimen	Duration (Weeks)	Day	Mosq. lot 340 (*P. falciparum*) No. mosq. applied	No. infected mosq. demonstrated by dissection	Day first parasites observed in blood	Clinical attack	Duration	Subsequent daily blood smears through months of:					
													Mar.	Apr.	May	June	July	Aug.
1	1298	Test	2/22	Day before	0.2 gm. 2× wk	4	2/23	5	3	3/4, 5, 6 only	None		*	Neg.	Neg.	Neg.	Neg.	Neg.
	1299	Control					2/26	5	3	3/7	Yes	4d interrupted + rec.						
2	1300	Test	2/23	Same day	0.2 gm. 2× wk	4	2/23	6	1	Neg.	None							
	1301	Control					2/26	4	1	Neg.	None							
3	1302	Test	2/24	Day after	0.2 gm. 2× wk	4	2/23	5	2	Neg.	None		Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	1303	Control					2/26	4	2	3/10	Yes	3d interrupted						
4	1304	Test	2/25	2 days after	0.2 gm. 2× wk	4	2/23	5	2	Neg.	None							
	1305	Control					2/26	5	2	Neg.	None							
5	1306	Test	2/26	3 days after	0.2 gm. 2× wk	4	2/23	5	3	Neg.	None		Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	1307	Control					2/26	5	3	3/8	Yes	4d interrupted						
6	1308	Test	2/27	4 days after	0.2 gm. 2× wk	4	2/23	5	2	3/8	None		*	Neg.	Neg.	Neg.	Neg.	Neg.
	1309	Control					2/26	4	2	3/9	Yes	3d interrupted + rec.						
7	1310	Test	2/28	5 days after	0.2 gm. 2× wk	4	2/23	5	3	Neg.	None							
	1311	Control					2/26	3	1	Neg.	None							

* All negative except as noted.

attained pyrogenic levels. The time of the initial dose of quinacrine in relation to the inoculation does not appear to have influenced the results.

Experiment B. Single Inoculation with *Plasmodium falciparum*

Purpose. To ascertain the period of time over which the administration of quinacrine hydrochloride should be continued, when given in two 0.1-gram doses on two discontinuous days a week, to protect against an inoculation with *P. falciparum* on a single occasion.

Material and Methods. Similar to those provided for experiment "A", with the exception that (a) only five pairs of patients were provided, (b) the initial dose of quinacrine hydrochloride was given to all test patients on the day subsequent to inoculation, and (c) the period over which quinacrine was given to the test patients varied from two to six weeks.

Results. The results of this experiment are presented in table 2. Pair no. 2 is rejected because the mosquitoes employed were not infected. Pair no. 3 is likewise rejected as the control patient did not develop an infection.

Clinically active infections developed in the controls to the first, fourth, and fifth pairs. No infection was detected in the corresponding test patients during the subsequent six months.

Conclusions. The regimen of quinacrine administered apparently prevented the development of infection in all of the test patients with satisfactory controls. Its continuation for a period of two weeks was sufficient.

These results suggest that in some instances the administration of quinacrine hydrochloride has prevented the acquirement of infection following a single inoculation, and has failed in this respect in other instances, although it appears that, in the latter instances, the infection was overcome shortly after becoming patent.

In view of the circumstance that the practical employment of this and other drugs to prevent infection is most common in areas where the prevailing epidemic or hyperendemic levels indicate that: (a) the risk of being nightly bitten by one or more infected mosquitoes is very real; (b) the risk extends over protracted periods of time; (c) this implies exposures to more than one species of parasite, and (d) also involves exposure to an unpredictable number of strains of each species as well, it did not appear that the two experiments described adequately reproduced the conditions of field exposure. In order to meet these requirements the following further experiments were performed.

Experiment C. Multiple Inoculations with Two Species of Parasites

Purpose. To ascertain the effectiveness of the administration of quinacrine hydrochloride, when given in daily doses of 0.1 gram for six days a week, against protracted inoculations with *P. vivax* and *P. falciparum*.

Material and Methods. The administration of quinacrine was begun one week prior to the initiation of inoculations.

Lots of mosquitoes separately infected with *P. vivax* and *P. falciparum* were provided and divided into batches containing three mosquitoes each. Batches were applied to patients for inoculation in alternation, so that each batch was utilized from two to three times with an interval for digestion between applications. All mosquitoes employed were finally dissected and examined for sporozoites. All inoculations were concurrent.

Three white male patients were placed on quinacrine one week before their inoculations began. Thereafter one received application of vivax-infected mosquitoes three times a week for four weeks, a second received application of falciparum-infected mosquitoes three times a week for four weeks, while the third received alternating application of vivax- and falciparum-infected mosquitoes six days a week for four weeks.

The details of the inoculations are given in table 3.

Results. Patient 559 received 19 inoculations with *P. vivax* in the 37 mosquito applications made during the fourth-week period. Parasites were encountered in the blood smears taken on December 22 and 23, and again on February 9 of the following year. Their numbers were scanty, they did not persist or increase, and no clinical activity became evident at these times. However, the patient finally exhibited a temperature of 102.6F. on March 25, and parasites were again detected on the following and subsequent days. This incubation was of 171 days duration counting from the first inoculation, or 147 days counting from the last. This initiated a clinical attack of vivax malaria which lasted for 34 days before it was interrupted therapeutically. The maximum

TABLE 2

Results of Experiment B

Pair	Patient's number	Rôle	Quinacrine hydrochloride: Day of initial dose	Quinacrine hydrochloride: Relation initial dose to inoculation	Quinacrine hydrochloride: Regimen	Quinacrine hydrochloride: Duration (Weeks)	Inoculation: Day	Inoculation: Mosq. lot 340 (*P. falciparum*) No. mosq. applied	Inoculation: No. infected mosq. demonstrated by dissection	Results: Day first parasites observed in blood	Results: Clinical attack	Results: Duration	Subsequent daily blood smears through months of: Mar.	Apr.	May	June	July	Aug.
1	1312	Test	2/23	Day after	0.2 gm. 2× wk.	2	2/22	8	1	Neg.	None		Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	1313	Control						4	1	3/10, 4/4	Yes							
2	1314	Test	2/23	Day after	0.2 gm 2× wk.	3	2/22	5	0									
	1315	Control						5	0									
3	1316	Test	2/23	Day after	0.2 gm. 2× wk.	4	2/22	5	1	Neg.	None							
	1317	Control						5	1	Neg.	None							
4	1320	Test	2/23	Day after	0.2 gm. 2× wk.	5	2/22	5	2	Neg.	None							
	1321	Control						5	1	3/10	Yes	3d interrupted	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
5	1318	Test	2/23	Day after	0.2 gm. 2× wk.	6	2/22	5	1	Neg.	None							
	1319	Control						4	1	3/8	Yes	4d interrupted	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.

parasite density of 3290 per c.mm. was observed on the ninth day of the parasitemia. The remission lasted 17 days and was followed by a recrudescence lasting five days which ceased spontaneously. Parasites were demonstrable as late as June 19.

Patient 560-1333 received, during the four-week period, 19 inoculations with *P. vivax* from the 36 mosquito applications, and 23 inoculations with *P. falciparum* from 36 further mosquito applications. *P. vivax* was first observed in the blood smear taken on December 23, and the clinical onset occurred on December 25. The incubation was of 109 days counting from the first inoculation,

TABLE 3

Details of Treatment C

Day	Quinacrine hydrochloride administered to each patient (grams)	Patient 559 P. vivax			Patient 560-1333 P. vivax-P. falciparum					Patient 1344 P. falciparum		
		Mosq. lot	Fed	Infect.	Mosq. lot	Fed	Fed	Infect.	Infect.	Mosq. lot	Fed	Infect.
Aug. 30	0.1											
31	0.1											
Sept. 1	0.1											
2	0.1											
3	0.1											
4	0.1											
5												
6	0.1	402	3	2	402	3		2				
7	0.1				400		3		2	400	3	2
8	0.1	402	3	0	402	3		3				
9	0.1				400		3		1	400	3	1
10	0.1	402	3	2	402	3		2				
11	0.1				400		3		2	400	3	1
12												
13	0.1	402	3	1	402	3		1				
14	0.1				400		3		3	400	3	0
15	0.1	402	3	0	402	3		1				
16	0.1				400		3		1	400	3	3
17	0.1	402	5	3	402	3		1				
18	0.1				400		3		3	400	3	1
19												
20	0.1	402	3	3	402	3		2				
21	0.1				400		3		3	400	3	2
22	0.1	402	3	1	402	3		2				
23	0.1				400		3		1	400	3	1
24	0.1	402	3	3	402	3		3				
25	0.1				400		3		3	400	3	2
26												
27	0.1	414	3	1	414	3		1				
28	0.1				412		3		1	412	3	1
29	0.1	414	2	2	414	3		1				
30	0.1				412		3		2	412	3	2
Oct. 1	0.1	414	3	1	414	3		0				
2	0.1				412		3		1	412	3	1

or 85 days counting from the last. The maximum parasite density of 37,900 per c.mm. was attained on December 29. Clinical activity had been continuous since the onset, but was interrupted on the 29th by the administration of 0.1 gm. quinacrine three times a day for five days. The last paroxysm occurred on the 31st, and the last parasites were observed on January 2. At no time was *P. falciparum* noted.

Patient 1334 received 17 inoculations with *P. falciparum* from 36 mosquito applications during the four-week period. He remained clinically and parasitologically negative except for an unidentified parasite noted in the smear taken on January 7, which is regarded as a contamination.

Conclusions. The quinacrine regimen employed has apparently given complete protection against the falciparum inoculations, but has not protected against the vivax inoculations.

Experiment D. Multiple Inoculations with *Plasmodium vivax*

Purpose. To ascertain the effectiveness of the administration of 0.1 gram of quinacrine hydrochloride when administered in daily doses for six days a week, against repeated inoculations with *P. vivax.*

Material and Methods. Lots of mosquitoes infected with *P. vivax* were provided and divided into batches of two mosquitoes each.

Two presumably susceptible white patients were provided. The administration of quinacrine to one was commenced one week before the first application of mosquitoes and discontinued one day subsequent to the last application of the mosquitoes. In the case of the other patients, the first administration of quinacrine was on the day of the first application of mosquitoes, and continued for one week after mosquito application terminated. Mosquito batches were applied in alternation, so as to allow an interval for digestion of the blood meal. Batches were applied to each patient on alternating days three times a week for four weeks. All mosquitoes employed were finally dissected and examined for sporozoites. The details are given in table 4.

Results. Patient 563 received a total of 24 inoculations with *P. vivax* from 24 applications of infected mosquitoes, during the four-week period. He exhibited a single parasite in the smear taken on December 8, but no others were seen until January 5, thereafter steadily increasing to a maximum density of 36,300 per c.mm. on January

TABLE 4

Details of Experiment D

Day	Patient 563				Patient 341			
	Quinacrine hydrochloride administered	Mosq. lot	Fed	Infected	Quinacrine hydrochloride administered	Mosq. lot	Fed	Infected
Oct. 31	(grams)				(grams)			
Nov. 1	0.1							
2	0.1							
3	0.1							
4	0.1							
5	0.1							
6	0.1							
7								
8	0.1	438	2	2	0.1	438	2	2
9	0.1				0.1			
10	0.1	438	2	2	0.1	438	2	2
11	0.1				0.1			
12	0.1	438	2	2	0.1	438	2	2
13	0.1				0.1			
14								
15	0.1	438	2	2	0.1	438	2	2
16	0.1				0.1			
17	0.1	438	2	2	0.1	438	2	2
18	0.1				0.1			
19	0.1	439	2	2	0.1	439	2	2
20	0.1				0.1			
21								
22	0.1	439	2	2	0.1	439	2	2
23	0.1				0.1			
24	0.1	439	2	2	0.1	439	2	2
25	0.1				0.1			
26	0.1	439	2	2	0.1	439	2	2
27	0.1				0.1			
28								
29	0.1	439	2	2	0.1	439	2	2
30	0.1				0.1			
Dec. 1	0.1	439	2	2	0.1	439	2	2
2	0.1				0.1			
3	0.1	439	2	2	0.1	439	2	2
4	0.1				0.1			
5								
6					0.1			
7					0.1			
8					0.1			
9					0.1			
10					0.1			
11					0.1			

12. The clinical onset on January 8 initiated a series of tertian paroxysms which continued as such until January 31, when they became quotidian. The incubation period was of 56 days duration counting from the first inoculation, or of 34 days counting from the last. The clinical activity ceased spontaneously on February 22. Parasites were subsequently observed as late as July 5.

When the experiment was begun, it was overlooked that patient 341 had previously been the recipient of malaria therapy on the service. He had been inoculated with the McCoy strain of *P. vivax* on August 3, 1938, with a clinical attack lasting 45 days subsequent to the onset on August 12 and thereafter experienced two recrudescences. It is to be expected therefore that he possessed an appreciable degree of homologous immunity.

Patient 341 received a total of 24 inoculations with *P. vivax* from 24 applications of infected mosquitoes during the four-week period. Parasites were observed in the smears taken on March 5 and 17; but they did not persist or increase in numbers, and no clinical activity was exhibited.

Conclusions. The quinacrine regimen employed in this experiment has not protected against the acquirement of a vivax infection, even in the case of the patient who possessed a homologous immunity, although the latter patient did not exhibit clinical activity.

Experiment E. Multiple Inoculations with *Plasmodium vivax*

Purpose. To ascertain the effectiveness of quinacrine hydrochloride when given in daily doses of 0.1 gram for six days a week in protecting against repeated inoculations with *P. vivax*, when a high blood level has presumably been secured by beginning drug administration two weeks before the initial inoculations (during the initial week giving the dose twice daily) and continuing the dosage for two weeks subsequent to the final inoculation.

Material and Methods. A lot of mosquitoes infected with *P. vivax* was divided into batches of two mosquitoes each.

Two presumably susceptible white male patients were provided. The administration of quinacrine to each was begun two weeks before the initial application of infected mosquitoes. Infected mosquitoes were applied on alternating days three times a week, and were used in alternation so as to allow an interval for the digestion

TABLE 5

Details of Experiment E

Day	Quinacrine Hydrochloride Regimen	Patient 590 Mosq. lot	Patient 590 Fed	Patient 590 Infected	Patient 591 Mosq. lot	Patient 591 Fed	Patient 591 Infected
Apr. 2	0.1 gm. 2 × day						
3	" " "						
4	" " "						
5	" " "						
6	" " "						
7	" " "						
8	" " "						
9							
10	" *once daily*						
11	" " "						
12	" " "						
13	" " "						
14	" " "						
15	" " "						
16							
17	" " "	478	2	1	478	2	2
18	" " "						
19	" " "	478	2	1	478	2	2
20	" " "						
21	" " "	478	2	2	478	2	2
22	" " "						
23							
24	" " "	478	2	1	478	1	1
25	" " "						
26	" " "	478	2	1	478	2	2
27	" " "						
28	" " "	478	2	2	478	2	2
29	" " "						
30							
May 1	" " "	478	3	3	478	2	2
2	" " "						
3	" " "	478	2	2	478	2	2
4	" " "						
5	" " "	478	2	2	478	2	1
6	" " "						
7							
8	" " "						
9	" " "						
10	" " "						
11	" " "						
12	" " "						
13	" " "						
14							
15	" " "						
16	" " "						
17	" " "						
18	" " "						
19	" " "						
20	" " "						

of the blood meal. All were finally dissected and examined for sporozoites. The administration of quinacrine was continued two weeks after the termination of the inoculations. The details are presented in table 5.

Results. Examination of daily blood smears taken from these patients until December 7 did not reveal a parasitemia. However, parasites were found in patient 590 on December 8, and these subsequently slowly increased in numbers to attain a maximum density of 9,600 per c.mm. on December 25. Clinical activity was initiated on December 14, and continued as a series of quotidian paroxysms until December 27, when the effect of therapeutic interference, begun the day before, was evident. The patient received 24 grains of quinine daily for seven days. The initiation of the parasitemia occurred 202 days after the termination of the quinacrine course and 217 days after the last application of mosquitoes. The incubation period was of 226 days duration counting from the first inoculation, or 208 days counting from the last. The daily smears from patient 591 remained negative until February 5, 1945, when one *P. vivax* was detected in the smear of that date. The numbers encountered in subsequent daily smears rose slowly to a maximum of 1850 on the 13th of February. The initiation of the parasitemia occurred *261* days after the termination of the quinacrine course, and *276* days after the last application of mosquitoes. The clinical onset occurred on February 10, after an incubation period of *300* days counting from the first inoculation, or *281* days counting from the last. The attack continued as a series of tertian paroxysms until its spontaneous termination on the 18th. Full therapy with quinine was initiated on the 22nd. It is interesting to note that on February 13 splenic palpation revealed that this organ had already attained the level of the umbilicus.

Conclusions. The extension of the quinacrine regimen two weeks in either direction beyond the period of the inoculation, has not prevented these patients from developing a clinically active vivax infection.

DISCUSSION

From the data submitted it appears that *P. vivax* and *P. falciparum* react differently when inoculated into white patients routinely receiving quinacrine. The observations of experiment C indicate that the administration of 0.1 gram six days a week continued during the period of exposure has afforded complete protection against the acquirement of a falciparum infection, but has not protected against a vivax infection. Even the materially lower dosages in the continued regimens employed in experiments A and B appear in some instances to have protected against the acquirement of falciparum infection, and in the instances when the infection became patent, appear to have exerted a therapeutic effect before the onset of clinical activity.

The observations of experiment D indicate that when the period of administration closely coincides with the time of exposure, doses of 0.1 gram per day for six days a week are inadequate to protect against the acquirement of a vivax infection.

Even when the period over which these doses are given is extended to at least two weeks before and two weeks subsequent to the time of exposure, vivax infections later become clinically active, although exhibiting a more extended incubation period. Since it is well known that vivax infections may, even without suppressive therapy, sometimes present protracted incubation periods approaching a year in duration, it is likely that a high proportion of individuals who have received suppressive therapy for extended periods will also finally experience clinically active vivax malaria after protracted incubation periods.

Attention should be directed to what would appear to be a fundamental difference in clinically active vivax infections arising on the one hand as the result of a single inoculation with a single strain of parasites, or on the other as a result of repeated inoculations with numerous strains. The attack arising from autochthonous infections acquired in regions of low endemicity or naturally induced infections are of the first category; those arising after protracted exposure in regions of high or hyper-endemicity are of the second. There is a possibility that the representatives of the different strains acquired under the latter circumstances do not simultaneously participate in the initial clinical activity, but may become successively and discontinuously active. In the latter event the various clinical episodes would not necessarily represent a series of recrudescences and relapses in which all strains present participate, but some at least may represent primary clinical activity due to distinct strains operating alone, and various recrudescences and relapses may be due to the reactivation of distinctly different strains.

OBSERVATIONS ON THE TRANSMISSIBILITY OF STRAINS OF PLASMODIUM VIVAX FROM PACIFIC WAR AREAS BY ANOPHELES QUADRIMACULATUS[1]

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INTRODUCTION

A considerable proportion of army personnel returning to this country from Pacific combat areas have malaria infections. In addition to soldiers requiring hospitalization for malaria *per se*, personnel hospitalized because of wounds, or other causes, are found to have malaria also. Parasitological studies of these patients indicate that about 90 per cent of these infections are with *Plasmodium vivax* (McCoy, 1944). However, in the field it is known that the ratio of *vivax* to *falciparum* infections is more nearly equal and often in favor of the latter parasite. This circumstance is not too surprising. In simultaneous inoculations with the two species, the *falciparum* infection develops first, and has clinical expression, apparently holding the *vivax* infection in abeyance. Later, sometimes after nearly a year, the *vivax* infection will develop and produce illness for the first time (Boyd and Kitchen, 1938). Infections with *falciparum* respond better to treatment than do *vivax* infections in the sense that intensive chemotherapy during the primary attack more often prevents relapses. In general, *falciparum* infections are short lived as compared with *vivax* infections, the latter tending to relapse over a period of two years, or more, in spite of any known form of therapy.

Since much of the war is being and will be fought in highly malarious areas, it is reasonable to infer that personnel of the armed services returning to this country will import numerous strains of malaria parasites, particularly of *P. vivax*. Inevitably personnel on furlough or discharged to civilian life will have malaria infections. Consequently, it is logical to expect that gametocyte carriers among these individuals will have a national distribution and that some of them will come in contact with anopheline mosquitoes in situations where malaria has been unknown for many years, as well as in places where malaria is endemic. The possibility of transmission of imported strains of malaria by indigenous anophelines, therefore, becomes a matter of considerable epidemiological significance and has stimulated the observations upon which this report is based.

The objective of this investigation was to determine whether Pacific strains of *P. vivax* can be transmitted by *Anopheles quadrimaculatus* under more or less optimum laboratory conditions. Through the courtesy of the administration of Kennedy General Hospital,[2] blood was taken from patients with *vivax* infections and used to inoculate patients on the malaria therapy service of Gailor Psychiatry Hospital. Upon the development of infections by these patients, lots of *Anopheles quadrimaculatus* were applied to them when gametocytemia favored infection of mosquitoes, as judged by experience with infections with the McCoy strain (Boyd, 1940) of *P. vivax*. Mosquitoes which had been fed were kept in a room with a temperature range of 76° to 82° F. and a mean relative humidity of 80 per cent. Stomach dissections were not done routinely, but salivary gland dissections were initiated on the 17th day of incubation. This date was selected because the exogenous cycle of McCoy *vivax* is completed uniformly in 17 days under the conditions given above.

Representatives of lots of mosquitoes which developed salivary gland infections were applied to patients until each patient had been bitten by at least three gland-positive specimens.

ORIGIN OF THE P. VIVAX STRAINS STUDIED

All strains studied presumably originated in Pacific war zones, the infections being acquired

[1]The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Tennessee.

[2]It is a pleasure to acknowledge the helpful cooperation of the hospital staff, particularly Lt. Col. Vernon E. Powell, Chief of Medical Service.

between January and April 1943. With one exception, the patients from whom the strains were obtained all denied having malaria prior to service in the Pacific areas and as civilians lived in urban communities north of the Ohio River. Strain P-4 was obtained from a native of Texas who gave a history of having had a febrile illness about 1932 which may have been malaria.

With one exception the patients had experienced from 3 to 8 relapses since the acute initial attack. Strain P-2 was obtained from a soldier who had received suppressive treatment with atabrine at the rate of 0.1 gram daily, including Sunday, from January 1 to April 1, 1943. He was then evacuated to the United States, reaching San Francisco on May 18. He stated that he had never been ill with malaria. Shortly after reaching Kennedy General Hospital early in June he developed a low grade fever, and a routine blood film was found to contain large numbers of *P. vivax*. On the date he was examined his blood contained 9,480 parasites per c.mm., of which 16 per cent were gametocytes. He was afebrile at this time and seemed to feel perfectly well. There was no demonstrable splenomegaly nor any finding of moment save a rather marked atabrine discoloration of the skin. Inoculation of a patient with blood from this individual produced a rather severe infection, so there is no question of the virulence of the strain. The soldier was from northern Michigan with a negative past history for malaria. Consequently, it would appear that suppressive treatment may have enabled him to establish a considerable tolerance to his infection.

TABLE 1

Some of the Characteristics of Blood-Induced Infections with Pacific P. vivax Strains

STRAIN	GAILOR HOSPITAL NUMBER	NUMBER PAROXYSMS	TEMPERATURE (RECTAL)		MAXIMUM PARASITEMIA PER CMM. BLOOD		REMISSION*	PREVIOUS MALARIA HISTORY
			Hours 103°+	Maximum	Asexual	Gametocytes		
P-1	88	9	89	107.2	27,600	6,348	T	None
	89	27	31	106.4	30,972	9,291	T	None
	127	12	85	106.4	21,434	4,287	S	Yes
	128	0	0		50–	0	S	Yes
	129	19	110	106.4	29,600	5.920	T	None
P-2	93	8	83	106.2	13,160	1,316	T	Doubtful
P-3	47	8	46	107.2	22,680	3,629	T	None
	119	0	0		50–	0	S	Yes
P-4	107	0	0		50–	0	S	Yes (vivax)
	142	5	19	106.0	13,680	8,208	S	Yes
	144	16	61	106.0	12,156	1,945	S	Doubtful
P-5	111	17	70	106.6	36,000	7,200	S	Yes
	114	18	42	106.6	10,212	102	S	Yes
P-6	51	13	76	106.6	14,960	1,795	T	Yes
	110	0	0		50–	0	S	Yes (vivax)
	147	17	101	106.6	29,600	7,104	T	Doubtful

* T — Induced therapeutically.
S — Spontaneous.

CLINICAL AND OTHER CHARACTERISTICS OF TROPHOZOITE-INDUCED INFECTIONS

Infection of four patients with negative and of three patients with doubtful past histories of malaria was accomplished without difficulty by inoculation with blood from infected soldiers at Kennedy Hospital. On the other hand, in nine cases with a definite past history of malaria, either induced or otherwise, four individuals failed to develop clinical activity and parasites appeared in their thick blood films on only one or two occasions (table 1).

In all patients with a negative past history of

malaria and in two of the three cases with a doubtful malaria history, artificial remission of the clinical course was resorted to. It appears likely that all cases with a therapeutic remission would have continued for some time had not quinine or atabrine been exhibited. A spontaneous remission of infection occurred in eight of the nine cases with a definite past history of malaria.

Regardless of previous malaria history, the clinical expression of infections with Pacific *vivax* strains was not unusual as compared with infection with McCoy *vivax*; in general the paroxysms were perhaps somewhat less severe. In only one instance was temperature in excess of 106.6°F. (rectal) recorded. In no instance was the parasitemia extraordinarily high, the maximum count being 36,000 per c.mm. All strains appeared to be good gametocyte producers. From these standpoints, the parasitological characteristics are not unlike those observed in McCoy *vivax*.

No detailed morphological studies were made of the parasites of the several strains isolated; however, one strain presented a rather obvious variation from the normal morphology of *P. vivax*. The youngest trophozoites seen resembled very closely those of *P. falciparum*: the rings were delicate and sometimes had two chromatin masses, multiple infection of red cells was common, and applique forms were seen. It was not until the development of ameboid forms and other morphological characteristics of *P. vivax*, some hours later, that the parasites could be identified definitely.

RESULTS OF ATTEMPTS TO INFECT A. QUADRIMACULATUS

In table 2 are shown the results of salivary gland dissections of mosquitoes fed on patients with Pacific *vivax* malaria, and other pertinent data. With regard to gametocytes, the values shown are for mature parasites as judged by morphology. Exflagellation studies were carried out at the time each lot of mosquitoes was fed, but the phenomenon was not demonstrated in a single instance tabulated. The number of male and female cells in every instance was approximately equal or slightly in favor of female gametocytes. If it is assumed that a level of 50 gametocytes per c.mm. is necessary to infect mosquitoes, it would appear that in every instance save one (P-26) gametocytemia favored mosquito infection.

While mosquito dissections were begun on the 17th day of the incubation period, they were not completed on this day. Often it was physically impossible to dissect all mosquitoes on this day; also the first mosquito dissections (strains P-1, 2 and 3) were negative, suggesting the possibility of a longer extrinsic incubation period than 17 days. Consequently, dissections were performed 17 to 39 days after mosquito lots were fed. This accounts for the discrepancy between the number of mosquitoes dissected and the number recorded as surviving 17 days (table 2). The difference

TABLE 2

Results of Salivary Gland Dissections of A. quadrimaculatus Applied to Patients with Induced vivax Infections, Pacific and McCoy Strains

STRAIN	NUMBER LOTS FED	GAMETOCYTES/CMM.		NUMBER MOSQUITOES		SPOROZOITES		INFECTION RATE PER CENT
		Mean	Range	Fed	Survived 17 Days	+	−	
P-1	12	1,072	281–2,463	554	385	0	290	0.0
P-2	1	1,316		34	15	0	13	0.0
P-3	2	1,679	1,664–1,695	101	38	0	34	0.0
P-4	2	1,200	672–1,728	100	61	12	7	63.1
P-5	4	999	344–1,747	218	129	12	25	32.4
P-6	3	817	21–1,436	136	117	11	40	21.5
McCoy	7	699	91–1,710	423	316	33	150	18.0

represents the number which died and which were autolyzed too badly for dissection.

No gland infections were demonstrated for strains P-1, P-2 and P-3. On the other hand, at least one mosquito in every lot of strains P-4, P-5 and P-6 was found to be positive for sporozoites. The infection rate of the latter lots, as a group, was 32.7 per cent as compared with a rate of 37.9 per cent for McCoy lots with gland-positive mosquitoes. However, the infection rate for all McCoy lots, that is including lots in which no gland-positive mosquitoes were found, was 18.0 per cent.

Data bearing on the quality of the gland infections are shown in table 3. From them it may be noted that less than a third (28.9

per cent) of the Pacific infections were heavy (+++ and ++++). Of the McCoy lots, slightly more than half (51 per cent) developed heavy gland infections. In this connection, it is interesting to note that the McCoy gametocyte values were the lowest recorded. This may indicate that the Pacific strains studied are less infectious to *A. quadrimaculatus* than McCoy *vivax*.

TABLE 3

Quality of Salivary Gland Infections of all Mosquitoes Recorded as Positive in Table 2

STRAIN	LOT NUMBER	GAMETOCYTES/CMM.	NUMBER INFECTED MOSQUITOES*				
			+	++	+++	++++	Total
P-4	47	672	2	0	0	0	2
	50	1,728	2	5	0	3	10
	2		4	5	0	3	12
P-5	37	1,053	0	1	0	0	1
	38	344	1	0	1	0	2
	39	853	2	1	1	1	5
	40	1,747	0	0	0	4	4
	4		3	2	2	5	12
P-6	42	994	1	0	0	0	1
	44	1,439	9	0	0	0	9
	49	21	1	0	0	0	1
	3		11	0	0	0	11
McCoy	43	100	1	2	0	0	3
	48	337	6	0	0	1	7
	51	91	2	2	1	6	11
	52	311	1	2	1	8	12
	4		10	6	2	15	33

* + = Very few sporozoites.
++ = Light infection.
+++ = Moderately heavy infection.
++++ = Very heavy infection.

Following sporozoite tramsmission of the Pacific strains, studies on the extrinsic incubation periods of P-5 and P-6 were made. It was found that the minimum incubation period is apparently 14 days, the same as for McCoy *vivax* under conditions of our laboratory.

Also, an attempt was made to determine the infectiousness of these patients to *A. quadrimaculatus* in relation to the development of the infection. Lots of 15 mosquitoes were applied to the patients with P-5 and P-6 infections, and to two patients with McCoy infections, applications being made daily from the time parasites appeared in the thin film regardless of the parasite count. As noted in table 4, the patient with P-4 malaria required treatment before clinical symptoms developed.

Table 5 records the results of dissections of these mosquitoes. It will be noted that it was possible to infect mosquitoes with P-5 (lot 53-E) even when gametocytes could not be demonstrated in blood films and that very low gametocyte values served to infect mosquitoes fed on patients with P-6 and McCoy infections. However, one of the patients with McCoy *vivax* failed to produce any mosquito infections although on one day (lot 57-F) the gametocytemia appeared particularly to favor mosquito infection. This difference in the relative infectiousness of patients coincides with the experience of Boyd (1942) with the McCoy strain.

TABLE 4

Sporozoite Transmission of Pacific vivax Malaria

STRAIN	PATIENT NUMBER	NUMBER MOSQUITOES APPLIED BY QUALITY OF GLAND INFECTION				LENGTH IN DAYS		NOTE
		+	++	+++	++++	Prepatent Period	Incubation Period	
P-4	193	0	2	0	1	10		(1)
P-5	186	0	0	0	4	12	13	(2)
P-6	168	4	0	0	0	16	17	
McCoy	180	1	1	0	2	12	12	

(1) This patient had a cerebral accident on the 7th day of the prepatent period, and quinine was exhibited when parasites appeared.

(2) Spontaneous remission after 4 paroxysms.

DISCUSSION

In this study it has been assumed that the *vivax* strains isolated are indigenous to situations where infection with them was acquired. This may be a false assumption. The writer knows of at least two instances of soldiers who acquired malaria infections during maneuvers in Louisiana and apparently suffered clinical relapses of these infections after reaching a combat area where malaria was hyperendemic. In both instances the time element ruled out infection in the combat

TABLE 5

Records of Dissection of Mosquitoes Applied on Consecutive Days Throughout Period of Parasitological Activity
Minimum Incubation Period 15 Days
Each Lot Originally Contained 15 Mosquitoes

STRAIN	LOT	PARASITEMIA/CMM.		RESULTS OF SALIVARY GLAND DISSECTIONS				TOTAL		
		Tropho-zoites	Gameto-cytes	+	++	+++	++++	−	+	Per cent positive
P-5	53-A	100–	0	0	0	0	0	13	0	
	53-B	288	0	0	0	0	0	5	0	
	53-C	2,203	45	1	0	1	0	11	2	
	53-D	1,200	0	1	0	0	1	8	2	
	53-E	5,760	0	6	3	2	0	4	11	
	53-F	2,726	114	0	0	0	0	10	0	
	53-G	480	0	3	3	3	2	3	11	
	7			*11*	*6*	*6*	*3*	*54*	*26*	*32.5*
P-6	56-A	1,594	102	0	0	0	0	8	0	
	56-B	1,340	116	1	1	0	0	5	2	
	56-C	7,728	672	4	0	0	0	11	4	
	56-D	8,026	334	2	1	0	1	11	4	
	56-E	1,591	0	0	0	0	0	13	0	
	56-F	2,652	0	1	0	0	0	14	1	
	56-G	1,326	0	0	0	0	0	15	0	
	56-H	1,856	0	0	0	0	0	15	0	
	56-I	16,072	328	0	0	0	0	15	0	
	56-J	3,762	38	0	0	0	0	14	0	
	56-K	2,200	0	0	0	0	0	10	0	
	56-L	100	0	0	0	0	0	10	0	
	12			8	2	0	1	141	11	7.8
McCoy	55-A	2,400	100	2	1	3	2	3	11	
	55-B	5,988	382	4	1	2	0	7	7	
	55-C	10,303	1,019	2	2	2	3	5	9	
	55-D	20,070	410	0	2	3	4	6	9	
	55-E	4,250	370	0	0	0	0	15	0	
	5			8	6	10	9	36	36	50.0
	57-A	100	9	0	0	0	0	5	0	
	57-B	2,673	111	0	0	0	0	10	0	
	57-C	4,600	0	0	0	0	0	8	0	
	57-D	3,514	146	0	0	0	0	13	0	
	57-E	2,226	142	0	0	0	0	17	0	
	57-F	5,266	718	0	0	0	0	10	0	
	57-G	450	0	0	0	0	0	12	0	
	7			0	0	0	0	75	0	0.0
	12			8	6	10	9	111	36	32.4

area. Consequently, it is not impossible that our troops carried *vivax* strains (perhaps McCoy among others) to Pacific war areas; that these strains were transmitted to noninfected individuals by indigenous anophelines; and that these secondary infections represent a portion of the infections in returning troops. However, the frequency of such infections, if they occur at all, must be relatively low.

That four of nine patients with histories of previous malaria infections, two of which are known to have been with *vivax*, failed to develop clinical infections after inoculation with Pacific *vivax* strains is surprising. This finding apparently indicates that a high degree of tolerance to infection with Pacific *P. vivax* strains is conferred by infections with *vivax* strains indigenous to this country. This is contrary to the general belief that tolerance conferred by a *vivax* infection is largely homologous. One is tempted to use the speculative epidemiological circumstance mentioned above as a possible explanation; but it is almost inconceivable that the four patients who did not develop clinical infections were all inoculated with *vivax* strains which originated in this country. There has been no opportunity to study this problem intensively. In this regard, it is hoped that data which are being accumulated by the armed forces will be analyzed with respect to the relative prevalence and subsequent course of malaria infections which occur among personnel originally from malarious areas of the United States for comparison with similar data for personnel from nonmalarious localities.

After the uniformly negative results of attempts to infect mosquitoes with strains P-1, P-2 and P-3, it was supposed that the mosquitoes were refractory to infection and the success of the experiments with the other three strains was not expected. In view of this later success, it seems necessary to take into consideration any factor which may have contributed to the failure to infect mosquitoes with strains P-1, 2 and 3. In this connection, it should be mentioned that the feeding of mosquito lots on patients with these strains were undertaken from July 2 to August 26; whereas the feeding of mosquitoes on patients with P-4, 5 and 6 infections began on August 27 and continued through October. The first lot of mosquitoes fed on a McCoy *vivax* patient was on September 1.

The summer of 1943 in Memphis was one of the hottest and driest on record, with mean daily temperature and humidity as follows:

	July	*August*	*September*
Temperature	83.7	84.0	70.4
Humidity, 12:30 p.m.	56	53	56

Most mosquito lots were fed between 10:00 a.m. and 2:00 p.m., and mosquitoes were exposed to atmospheric temperature for a period of about 30 minutes after taking an infected blood meal. It is conceivable, on the basis of the work of Stratman-Thomas (1941), that the high atmospheric temperatures and low relative humidities to which lots of mosquitoes were exposed during July and August may have interfered with the development of the extrinsic cycle. It is unfortunate that comparative studies with McCoy *vivax* were not possible at the time the first three strains were being studied.

From both a qualitative and a quantitative standpoint, the salivary gland infections with Pacific strains 4, 5 and 6 do not compare unfavorably with McCoy strain infections on the basis of the observations reported here. Moreover, it would appear that very low densities of gametocytes may serve to infect mosquitoes. With this in mind, particular attention is drawn to the circumstances mentioned with reference to the origin of strain P-2. The soldier from which the isolation was made had an asymptomatic parasitemia of high grade and 16 per cent of the parasites were gametocytes. Other factors being favorable, such an individual could serve as an excellent infector of mosquitoes. There must be very many similar cases now, and the number will probably increase in time.

The findings reported would seem to indicate the advisability of feeding mosquitoes on soldiers with parasitemia in an effort to appraise more directly the probability of the influence of gametocyte carriers on the epidemiology of malaria in this country.

SUMMARY

From soldiers who had presumably acquired their infections in Pacific war areas, six strains of *P. vivax* were isolated by transfer of blood to patients who were candidates for malaria therapy. The clinical and parasitological characteristics of the induced infections were not unlike those of infections with McCoy strain of *P. vivax*, the strain of reference used throughout the study.

Some of the patients with known or probable histories of former *vivax* infections demonstrated considerable immunity to the Pacific strain infections, four patients being refractory.

When gametocytemia of these induced infections seemed to favor mosquito infection, *A. quadrimaculatus* mosquitoes were fed. Subsequent to a minimum incubation period of 17 days, these mosquitoes were examined for sporozoites. Salivary gland infections were demonstrated in mosquitoes fed on patients with infections with three of the six strains. Sporozoite transmission of these three strains was accomplished. Subsequently the infectiousness to *A. quadrimaculatus* of patients with two of these strains was studied. It was found that salivary gland infections sometimes developed following the feeding of mosquitoes on patients with submicroscopic gametocytemia.

It is concluded that at least some of the *vivax* strains from the Pacific war area are capable of being transmitted by *A. quadrimaculatus*. It is believed that this mosquito is a somewhat less efficient vector for these strains than for McCoy *vivax*.

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MALARIA IN THE AMAZON VALLEY OF BRAZIL DURING 1942 AND 1943[1]

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Malaria is generally suspected of being the principal retarding factor in the economic development of the Amazon Basin. Although the incapacitating effect of epidemic and endemic malaria is evident in many localities throughout the valley, the actual prevalence of the disease has not been known. One of the first activities of the *Serviço Especial de Saude Publica* under the joint auspices of the Institute of Inter-American Affairs and the Brazilian Government was the estimation of the incidence of malaria in the principal centers of population as determined by examination of blood samples.

THE AMAZON VALLEY

Size. The Brazilian portion of the Amazon Valley extends over the northern half of Brazil including about 3,800,000 square kilometers. The Valley is a shallow elliptical basin the outer Brazilian boundaries of which are latitude 5° north and 17° south, and longitudes 47° and 74° west. The Amazon-Solimões River, including its tributaries the Rios Negro, Madeira, Tapajoz, and Xingú, and the adjoining Pará-Tocantins system, regularly overflow during the rainy season inundating vast territories. Even in the dry season in the State of Pará much of the inhabited land along the rivers is periodically flooded at high tide. The entire valley is remarkably flat as shown by the fact that Belem near the coast is about 14 meters above sea level, while Iquitos, Perú, 3200 kilometers up the valley is at an elevation of only 100 meters.

Terrain. The terrain of the valley is usually described under two categories, the *varzea* or flood land, and the *terra firme*. The flood land is in a constant state of formation and evolution with each stage showing a characteristic flora. At the water's edge there are the succulent grasses commonly called *canaranas*, and the *cerainas* (*Salix martiana* and *Alchornea castaneifala*). Immediately inland are the big leaved *imbaubas* (*Cecropia paraensis*). Beyond these are the numerous varieties of palms. The *terra firme* includes the *campos* or prairie lands, and the forested plateaus and hills.

Seasons. Generally speaking there is a wet and a dry season throughout the whole area. Even in the driest months there is some rain, however, and the months with little rainfall are fewer than is the case in most tropical countries. The months with the lowest rainfall in Manaus are July, August and September, with an average of 54 mm., while in Belem October and November are the driest months with an average of 87 mm. The greatest rainfall in Manaus occurs from December through April averaging 253 mm. monthly, and in Belem from January through May averaging 377 mm., (Brasil, 1942). The average yearly rainfall for Manaus over a period of 24 years was 1995 mm. with rain recorded on an average of 167 days per year. In Belem during a period of 12 years the average yearly rainfall was 2805 mm. with an average of 250 rainy days per year.

Temperature. The temperature range is small. In Manaus the absolute maximum during a period of 24 years was 37.8°C. in October, and the absolute minimum of 17.6°C. occurred in July. In Belem the absolute maximum during 12 years' observations was 35.1 in August, while the absolute minimum of 18.5 was recorded in July. The average daily temperature ranged between 25.7 and 27.6°C. in Manaus, and between 24.9° and 26.2°C. in Belem.

Population. The latest census taken in 1940 records a population in the Amazon Valley of about 1,500,000 persons, most of whom live near the navigable rivers. The number of uncivilized Indians that inhabit the hinterland is not known.

MALARIA STUDIES

The studies on malaria reported in this paper were made principally in the towns situated on the waterways of the valley and in the farming country between Belem and Bragança. The inhabitants along the river live in huts built on stilts above

[1]The studies herewith reported were part of the program of the *Serviço Especial de Saude Publica* maintained jointly by the Ministry of Education and Health of Brazil and the Institute of Inter-American Affairs.

the mud and water, or in small villages on the high knolls surrounded with water, or in towns built on the upland plateaus.

In very few places do the people attempt to grow their own food, consequently there is usually a food shortage which in conjunction with the heavy intestinal parasite infestation causes severe malnutrition throughout the valley. These conditions are important factors in lowering the resistance of the inhabitants not only to malaria but to other diseases as well.

Technique. A study of the rainfall curve for Belem indicated that the highest malaria rate would probably occur in May or June and the lowest in December. Based on this assumption parasite surveys were undertaken in December 1942 and in June 1943. Thick blood films were taken from a predetermined sample of the population in towns of the valley which are accessible by air, boat or train transportation. A sample of about 6 per cent was taken in Belem, about 20 per cent from the small towns with more than 1000 inhabitants, and 30 per cent or more from the towns with less than 1000 inhabitants. In a few instances the official census was lower than the actual number of persons examined because of the temporary influx of persons from surrounding regions. The blood films were shipped to Belem where they were stained by Giemsa according to the method of Freitas (1942) and examined 10 minutes per slide. Even if malaria parasites were found during the first few minutes of examination, the search was continued throughout the entire ten minutes to detect the possible presence of other species of plasmodia.

Results. Belem, situated on the Pará River, with a population of 177,100 is the largest city in the valley. In the December survey a sample of 9831 persons was examined about one third of whom were males. This sex ratio resulted from the method of survey which was a house to house canvas along selected streets during the usual daylight working hours when employed males were absent. In the age group under one year very few examinations were made because of the reluctance of mothers to allow the infants to be bled. The children 1 to 9 years of age and the group 10 to 19 years of age each constituted about one quarter of the total examinations while the remaining one half included the adult group 20 years old and above. The parasite infection rates correspond to the expected trend in showing somewhat higher rates in children and progressively lower rates as age advances. There was also a slight difference in rates when analyzed by sex. Of the total persons examined 363 or 3.7 per cent showed malarial parasites, the rate for males being 4.7 and for females 3.2 per cent. Among the infants 6.6 per cent were positive. The children one to nine years of age showed a rate of 4.7 per cent; the young people aged 10 to 19 years showed a rate of 3.8 and the adults over 20 years of age a rate of 2.9 per cent. Of the total 363 infected individuals 66.4 per cent harbored *Plasmodium vivax* and 33.9 per cent harbored *Plasmodium falciparum*. The *vivax* cases showed a higher rate of gametocyte carriers than the *falciparum* cases, the rates being respectively 43.2 and 26.0 per cent.

Five towns in the agricultural district along the railroad between Belem and Bragança were sampled. The populations ranged from 1900 to 5700. The infection rates were uniformly low with very little variation in either the sex or age groups with the notable exception of the 44 infants under one year all of whom were negative for malaria. The parasite rates by towns were as follows: João Coélho 2.2; Castanhal 1.5; Igarapé-Assú 0.5; Capanema 4.2; Bragança 1.5.

The towns studied on the Pará River showed for the most part higher rates, especially among the males. The parasite rates were as follows: Vigia near the sea, 5.7; Abaetetuba near the mouth of the Tocantins 2.8 and Curralinho in the straits between the Amazon and the Pará, 8.9. These villages are alike in being situated near areas subject to daily tidal inundations and where monthly maximum tides create numerous breeding places for Anophelines.

On the Tocantins river four towns were selected for study, and the following rates were observed: Cametá 6.3; Baião, 7.3; Alcobaça, 11.0 and Marabá 11.1.

The four towns surveyed on the Amazon proper showed a greater diversity in infection rates. Gurupá with an average rate of 16.1 per cent was one of the three most malarious places surveyed in the valley at this time. The town of Monte Alegre on the river showed a rate of 5.7 while the neighbouring inland village of Mulata was experiencing a malaria epidemic with a rate of 72.9 per cent among the persons examined. Itacoatiara and Manaus were inadequately sampled but the few slides taken showed rates of 2.2 and 12.3 per cent respectively.

On the Madeira River, Porto Velho showed a malaria infection rate of 5.7 per cent. On the Purús River, Labrea showed a rate of 7.0 per cent, while Bôca do Acre and Rio Branco on the River Acre showed the low rates of 1.4 and 0.8 per cent.

The only town surveyed on the Rio Branco was Bôa Vista which showed a rate of 16.4 per cent.

A summary of these results on 19,629 persons from all 23 towns and villages is given in table 1, showing infection rates by sex, age groups and species of parasites. A total of 979 positive smears or an infection rate of 5 per cent was found.

A second survey was undertaken in June 1943 at the end of the wet season. Most of the towns investigated in 1942 were reexamined and several other towns were surveyed for the first time. In Belem 10,558 blood samples were examined of which 361 or 3.4 per cent were positive for malaria parasites.

All towns investigated on the Bragança railroad showed low rates as follows: João Coêlho 1.5; Castanhal 0, Igarapé-Assú 0.4, Capanema 0.6 and Bragança 0.3 per cent.

At the mouth of the Pará river Curuçá on the mainland and Soure on the Island of Marajó showed parasite rates of 0.3 and 0.7 per cent respectively. Vigia showed a rate of 7.2 per cent, Mojú was experiencing an epidemic with 41.7 per cent, Abaetetuba showed 0.2 per cent, Salvaterra 2.2 and Curralinho 2.9 per cent. Breves also experienced an epidemic during June showing a rate of 22.0 per cent. On the Tocantins, Cametá, Baião, Alcobaça and Marabá showed rates of 2.0, 1.5, 1.9 and 4.3 per cent respectively, while Mocajuba with only 75 persons examined had a rate of 10.7 per cent.

On the Amazon proper five towns were examined with rates as follows: Gurupá 0.5, Santarem 0.8, Parintins 2.9, Itacoatiara 1.7 and Manaus 3.2 per cent. Maués on the River Maués gave a rate of 1.3 per cent. On the River Solimões, Fonte Bôa showed 0, Tefé 1.3, and Tabatinga 0 per cent.

On the River Madeira, Humaitá and Porto Velho showed blood parasite rates of 2.3 per cent. The four towns examined on the Purús River and

TABLE 1

Malaria Survey in the Amazon Valley at End of Dry Season by Age Groups, Sex and Species of Parasites, in December 1942

AGE GROUPS	NBR. EXAMINED			NBR. POSITIVES				SPECIES		GAMETOCYTES	
	Male	Female	Total	Male	Female	Total	% positive	Falciparum	Vivax	Falciparum	Vivax
–1	84	82	166	4	5	9	5.4	2	7	0	5
1–9	2632	2508	5140	204	162*	366	7.1	136	230	51	130
10–19	2000	3333	5333	129	128	257	4.8	110	146	44	79
20+	2508	6482	8990	134	213*	347	3.9	159	190	55	75
Totals:.....	7224	12,405	19,629	471	508	979	5.0	407	573	150	289

* One case of *P. malariae* included.

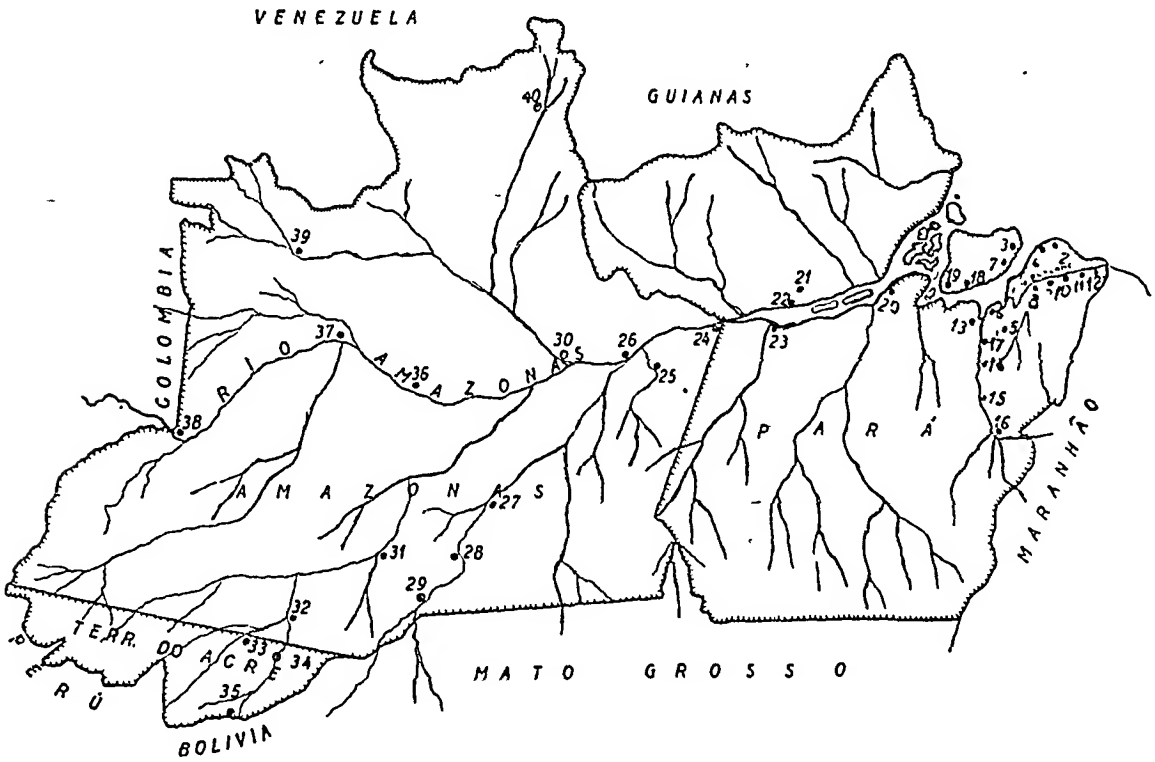
TABLE 2

Malaria Survey in the Amazon Valley at End of Wet Season by Age Groups, Sex and Species of Parasites in June 1943

AGE GROUPS	NBR. EXAMINED			NBR. POSITIVES				SPECIES		GAMETOCYTES	
	Male	Female	Total	Male	Female	Total	% positive	Falciparum	Vivax	Falciparum	Vivax
–1	71	77	148	1	3	4	2.7	1	3	0	3
1–9	3672	3635	7307	159	149	308	4.2	147	163	73	102
10–19	3000	4545	7534	115	142	257	3.4	113	145	60	75
20+	2207	7283	9490	101	186	287	3.0	132	155	55	67
Unknown	1053	1560	2613	15	19	34	1.3	19	15	10	8
Totals.	10,003	17,100	27,103	391	499	890	3.3	412	481	198	255

upper tributaries showed parasite rates as follows: Labrea 9.2, Bõca do Acre 0.5, Sena Madureira 0.6, Brasiléa 3.7 and Rio Branco 1.4 per cent. São Gabriel on the Rio Negro and Bôa Vista on the Rio Branco showed rates of 4.0 and 9.1 per cent respectively.

These results on 27,103 persons from 37 towns are tabulated in table 2 showing infection rates by sex, age groups and species of parasites. The 890 positive smears represent an infection rate of 3.3 per cent. The localities studied in both surveys are shown on Map I.



MAP I

Amazon Valley

Distribution of Towns surveyed for Malaria incidence in December 1942 and June 1943

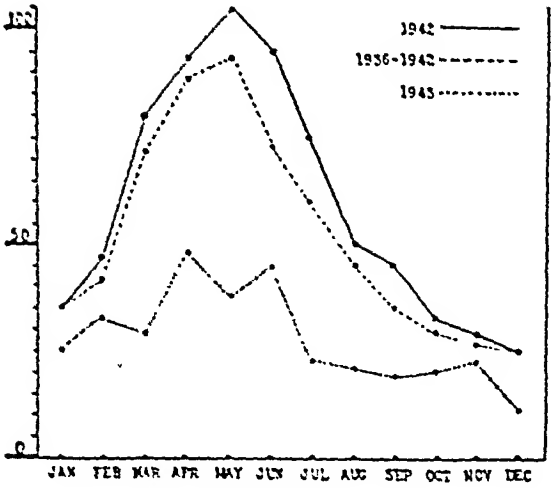
Towns

1. Belem
2. Curuca
3. Soure
4. Vigia
5. Moju
6. Abaetetuba
7. Salvaterra
8. Joao Coelho
9. Castanhal
10. Ig. Assu
11. Capanema
12. Braganca
13. Cameta
14. Baiao
15. Alcobaca
16. Maraba
17. Mocajuba
18. *Curralinho*
19. Breves
20. Gurupa

Towns

21. Mulata
22. Monte Alegre
23. Santarem
24. Parintins
25. Maues
26. Itacoatiara
27. *Manicore*
28. Humaita
29. Porto Velho
30. Manaus
31. Labrea
32. Boca do Acre
33. Sena Madureira
34. Rio Branco
35. Brasilea
36. Tefe
37. Fonte Boa
38. *Tabatinga*
39. Sao Gabriel
40. B. V. Rio Branco

A study of the records of *Santa Casa de Misericordia do Pará* shows that the supposition that the lowest malaria rate would occur in December and the highest rate in May or June was well founded. Graph I shows average monthly cases of malaria diagnosed in the *Santa Casa* laboratory



GRAPH I

MALARIA IN BELEM. POSITIVE CASES DIAGNOSED IN STA. CASA

by positive smears during the period 1936 to 1942, and cases by months for the years 1942 and 1943. The greatest number of cases occurred in May and the lowest in December. The December survey in Belem should therefore show the incidence of malaria when the fewest cases are present. The June survey should normally show a higher rate. It can be seen from Tables I and II that instead of an increase in malaria in June above the December incidence of 5.0 per cent there was an actual decrease to 3.3 per cent. It should be pointed out, however, that the first survey was made before any appreciable malaria or mosquito control was undertaken. The survey in June on the other hand was made after large quantities of atebrine had been distributed throughout the valley and anti-mosquito work commenced in many of the localities. There is also the possibility that the reduction in incidence was due in part to a malaria cycle of several years duration similar to that which occurs in the southern United States. There is some indication that such a cycle exists in the Amazon Valley and that it is operating in many of the areas where *A. darlingi* is always present. In the extensive regions where *A. darlingi* is transitory this cycle cannot exist, as it can function only when the mosquito factor is constant, leaving the immunity factor in the human population as the only variable.

Anopheles darlingi is the principal malaria vector in the Amazon Valley and the only Anopheline, with the exception of the coastal species *Anopheles aquasalis*, capable of producing epidemic malaria in the region (Deane, Causey and Deane in press). *Anopheles darlingi* breeds most prolifically during, and immediately following the rainy season, and succeeds in passing the dry season only in those areas where there are permanent lakes or ponds from which it again disperses to the surrounding country with the beginning of the rains.

In localities where the resistant foci of *A. darlingi* occasionally dry up due to a particularly long dry season, the entire *darlingi* population may die out. The duration of this malaria vector's absence then depends upon the proximity of other resistant foci, or the chances of reintroduction by transportation. Some areas have been free of *darlingi* for several years as shown by the lack of endemic malaria in the population. When such areas become reinfested with *darlingi* the relatively non-immune population suffers from severe epidemic malaria.

The fact that *A. darlingi* is limited in its distribution during the dry season to large bodies of water or swampy regions many of which can be drained or effectively treated with Paris green or oil, makes this mosquito highly vulnerable to eradication. The most economical method of combating malaria in many regions of the Amazon Valley is therefore by the eradication of the vector from the dry season breeding areas in the proximity of the towns and villages that are to be protected.

SUMMARY

This is a report of two malaria parasite surveys made in the Amazon Valley during 1942 and 1943. The survey in December 1942 on 19,629 persons was made at the end of the dry season when the prevalence of malaria was expected to be lowest, and before control or prophylactic measures were undertaken. The survey in June 1943 on 27,103 persons from 37 localities was made at the end of the wet season when malaria incidence might be expected to be greatest. A study of hospital records on malaria cases in Belem before 1942 supported this assumption as to seasonal prevalence. The incidence of 3.3 per cent in June after control measures were instigated was shown to be lower than the incidence of 5.0 per cent at the expected low season in December.

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PERSISTENCE OF YELLOW FEVER VIRUS IN MOSQUITOES AFTER DEATH OF THE INSECT[1]

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The isolation of virus from captured wild mosquitoes constitutes an important phase in the epidemiological investigation of jungle yellow fever. In this connection 2 points were presented for laboratory investigation: (1) whether mosquitoes which had died in transit to the laboratory could be used for the isolation of virus, and (2) whether the methods used to kill mosquitoes which were received alive at the laboratory had any deleterious action on yellow fever virus.

MATERIAL AND METHODS

The mosquitoes used were of the species *Aedes* (*Stegomyia*) *aegypti* (Linnaeus), *Haemagogus equinus* (Theobald), and *Haemagogus spegazzinii* (Brethés). The first 2 species were maintained in colonies. The *Aedes aegypti* stemmed from larvae captured in Nova Iguassú, Rio de Janeiro. The *Haemagogus equinus* were offspring of the colony established by Dr. Ernest Osorno—Mesa at Bogotá, Colombia. The *Haemagogus spegazzinii* were always first generation descendants of wild mosquitoes captured in the States of Bahia or Mato Grosso. The insects, chosen at random were infected through feeding on marmosets or *Cebus* monkeys at a time when virus was circulating in the blood. At the conclusion of each feeding period, either a sample lot of 5 engorged mosquitoes was emulsified and inoculated into mice in order to check the presence of virus, or the animal was bled and the actual amount of virus in the blood was determined by titration, using serial tenfold dilutions of serum, injected intracerebrally into Swiss mice. One group of 6 mice was used for each dilution. The technique used for breeding, storing and infecting the mosquitoes was essentially that described by Stokes, Bauer, and Hudson (1).

Two jungle strains of yellow fever virus were employed, the Olympio Christo (O.C.) and the João Zanabrea (J.Z.). The pathogenicity of these 2 strains has been studied by Laemmert (2).

After death the insects were kept at a temperature of 26 to 30.5°C. Determinations of the virus content of the mosquito bodies were made at various time intervals. The mosquitoes were triturated in a diluent consisting of 10 ml. of a human serum, known to be free from yellow fever antibodies, in 90 ml. of 0.9 per cent NaCl solution. Unless otherwise stated a volume of 0.18 ml. was used for each insect. The emulsions were centrifuged, and the supernatants were injected intracerebrally into Swiss mice 21 to 27 days of age. When tests for the presence of virus were to be made, 0.03 ml. of the undiluted supernatant was injected into each of 6 mice. When a quantitative estimation of the virus content was required, serial tenfold dilutions of the supernatant were injected. A group of 6 mice was used for each dilution. The inoculated mice were kept under observation for 21 days. Those showing atypical or questionable signs of illness were sacrificed. Their brains were removed and used for subinoculation into groups of 6 mice 21 to 27 days of age. At the end of the observation period the surviving mice were tested for immunity by the intracerebral inoculation of a dilution of French neurotropic yellow fever virus containing about 500 LD_{50} for mice. The challenge inoculum was always titrated intracerebrally in 21- to 27-day-old mice.

Calculations of "50 per cent mortality end-point titers" were made by the method of Reed and Muench (3).

EXPERIMENTAL

Tests with chloroform

Mosquitoes to be killed by chloroform were placed, in lots of 5, in capture tubes 12 cm. long and 3 cm. in diameter. A thin cotton plug was placed in the funnel-shaped orifice at the end of the tube, and chloroform was dropped onto this

[1] The work on which these observations are based was carried out under the auspices of the Serviço de Estudos e Pesquisas sôbre a Febre Amarela (Yellow Fever Research Service), which is maintained jointly by the Ministry of Education and Health of Brazil and the International Health Division of The Rockefeller Foundation.

plug. Mallinckrodt's "technical" grade of chloroform was used.

Preliminary tests showed that 1 drop of chloroform, the amount routinely used in this laboratory, produced immobilization for a period of about 2 hours but did not kill the insects. Neither did it diminish, significantly, their virus content. If this amount was increased to 2 or 3 drops the mosquitoes succumbed promptly, but the virus activity decreased very rapidly. Following this preliminary test a quantitative measurement of the rate of virus loss was made.

Tests with potassium cyanide

Experiment 2. The mosquitoes used in this test were infected at the same time and from the same marmoset as were those which were used in Experiment 1. The titrations were made simultaneously with those of the previous test. Groups of 5 mosquitoes were put into capture tubes, and the uncorked end of a potassium cyanide killing tube was placed in contact with the unplugged end of the capture tube until the insects were stunned. The cyanide tube was then introduced into the capture tube for a few moments. The mosquitoes died quickly. Titrations of the virus content of the mosquito bodies, always in groups of 5, were made at various time intervals, as shown in table 1. The titer was practically unchanged for 8 hours and was not significantly lowered until 20 hours had elapsed. Small quantities of virus were recovered up to 45.5 hours after the death of the insect.

TABLE 1

Rate of virus loss in Aedes aegypti killed by potassium cyanide, tobacco smoke, chloroform, and ether

HOURS	50 PER CENT MORTALITY END-POINT TITERS AT INTERVALS AFTER DEATH OF MOSQUITO LETHAL AGENT			
	Lot 1			Lot 2
	Potassium cyanide	Tobacco smoke	Chloroform	Ether
0	$10^{-4.25}$	$10^{-4.15}$	$10^{-4.25}$	$10^{-4.35}$*
¼	—	—	$10^{-4.05}$	$10^{-4.15}$
½	—	—	$10^{-1.55}$	$10^{-4.40}$
1	$10^{-4.30}$	$10^{-4.35}$	0	$10^{-2.60}$
1½	—	—	0	—
2	$10^{-4.60}$†	$10^{-4.20}$	0	$10^{-4.25}$
4	$10^{-3.60}$	$10^{-3.25}$	—	—
8	$10^{-4.10}$	$10^{-4.20}$	—	—
16	$10^{-3.20}$	$>10^{-3.80}$	—	—
20	$10^{-2.20}$	0	—	—
24	$10^{-1.80}$	$10^{-2.20}$	—	—
28	$10^{-2.20}$ or >	0	—	—
32	0	0	—	—
40	$10^{-0.80}$	0	—	—
45½	$10^{-0.80}$	0	—	—
48	0	0	—	—

* A control group of 5 mosquitoes killed by potassium cyanide gave $10^{-4.65}$ titer.

† This original mosquito emulsion contained $\frac{1}{11}$ instead of $\frac{1}{6}$ of a mosquito per 0.03 ml. of suspension.

— indicates that no test was made.

0 indicates that all mice survived the original inoculation but succumbed to a challenge inoculation.

Experiment 1. Aedes aegypti were allowed to feed on a marmoset infected with the Olmpio Christo strain of yellow fever virus. Inoculation of a suspension of freshly engorged insects showed that the virus content of the ingested blood was satisfactory. Thirty-six days later the mosquitoes were divided into groups of 5 and were killed with 3 drops of chloroform. The virus content of the groups of 5 insects was determined at various time intervals after death. The results are shown in table 1. Most of the virus was destroyed within 30 minutes, and all infectivity had been lost at the end of 1 hour.

Tests with tobacco smoke

Experiment 3. The mosquitoes used in this experiment were also infected at the same time, and from the same marmoset, as were those used

in Experiment 1. The titrations were carried out simultaneously with those of the previous tests. Mosquitoes, in groups of 5, were placed in capture tubes into which smoke from a local brand of cigarettes was blown, repeatedly, through a pipette. The virus content of the mosquito bodies was estimated as previously described. There was no significant fall in virus content for 8 hours, and the titer was not significantly lower until 20 hours after death. No virus was demonstrable after 28 hours. The results are shown in table 1.

Tests with ether

Experiment 4. The *Aedes aegypti* employed in this test had been infected 72 days previously by feeding on a marmoset infected with the Olympio Christo virus. The virus content of the serum was such that a dilution of $10^{-7.50}$ contained an infective dose for mice. The mosquitoes were placed in capture tubes, in groups of 5, and killed by the application of 20 drops of ether. Mallinckrodt's "U.S.P. IX" grade of ether was used. At intervals thereafter sample lots of 5 mosquitoes were emulsified and inoculated into groups of mice. Virus in a quantity sufficient to kill or to immunize all of the 6 mice in each group was present up to 4 hours. Sufficient was present to kill 1 of 6 mice at 8 and 28 hour intervals. No virus was recovered at 16, 20, or 24 hours.

Experiment 5. The *Aedes aegypti* used in this test had been infected 80 days prior to use, by feeding on a *Cebus* monkey infected with the João Zanabrea strain of virus. The titer of virus in the serum at the time of feeding was $10^{-5.00}$. The experiment was similar to Experiment 4, except that quantitative titrations of the virus content were made. The results are presented in table 1. Disregarding the anomalous result obtained with insects which had been dead for 1 hour, there seemed to be no significant loss in virus content up to the end of 2 hours.

Tests with insects dying of starvation

Experiment 6. The *Aedes aegypti* used in this experiment had fed on a *Cebus* monkey circulating the João Zanabrea strain of virus. The titer of virus in the serum was $10^{-7.20}$. All food and water were removed from the mosquito cage 72 days after the infective meal. The insects were examined at hourly intervals during the day, and all of the dead or obviously weak individuals were removed. The weak mosquitoes were examined at frequent intervals, and the exact time of their death was noted. For the portion of the remainder which died during working hours, the time of death is known within 1 hour. Mosquitoes dying overnight were grouped, and the time of their deaths is known only within a 16-hour range. The quantity of diluent used was 0.35 ml. for 1 mosquito, 0.18 ml. for each mosquito in lots of from 2 to 5, and a fixed volume of 0.9 ml. for lots of 5 to 10 insects.

Virus in a quantity sufficient to kill or to immunize all of the 6 mice was recovered repeatedly from mosquitoes dead for 1, 2, 4, 5, and 6.5 hours. It was also recovered in a quantity sufficient to kill 3 mice of a group of 6 inoculated with an emulsion made from 1 mosquito which had been dead for 17.5 hours. One mouse of a group inoculated with an emulsion of 8 mosquitoes which had been dead for a period between 25 and 41 hours also succumbed.

Tests with insects dying of "natural causes"

Experiment 7. To test the practical application of the results obtained in Experiment 6 the following test was made. Mosquitoes of the species *Haemagogus equinus* and *Haemagogus spegazzinii*, which had ingested traces or larger amounts of either of the virus strains, and which died, spontaneously, during the course of transmission experiments, were employed in these tests. A search for dead mosquitoes was made only at intervals of 1 to 3 days. Therefore, the exact time of death of the insects is unknown. The majority of tests were made with suspensions containing 1 mosquito only. The remainder of the emulsions contained from 2 to 8 specimens.

Virus was recovered with equal ease from lots which contained 1 or several insects. It was detected in 1 of 3 lots which had died sometime within 72 hours preceeding injection. Among 20 lots which had died within 48 hours prior to testing, 8 lots contained virus in an infective quantity. When tested within 24 hours after death of the insects, virus was demonstrable in 33 of 47 lots.

DISCUSSION

It is apparent from the results presented that the use of chloroform to stun insects which are to be used in attempts to isolate yellow fever virus is inadvisable. When employed in the minimal effective concentration it is innocuous to the virus. However, this critical concentration is easily overstepped.

The use of potassium cyanide is very effective, and it is non-injurious to virus. Unfortunately,

this method of killing insects is cumbersome and time consuming. The same objections apply to the use of tobacco smoke which, in addition, is relatively ineffective. The use of ether, then, would appear to be the method of choice, since ether is easy of application and does not significantly lower the virus activity within the period of time required for inoculations.

The results obtained with insects dying of starvation and "natural causes" demonstrate that mosquitoes which do not survive transportation to the laboratory may still be used in tests for the presence of virus. Similarly, insects which die during the course of transmission experiment, and, which ordinarily would be discarded, may be tested for virus. This results in sparing the lives of hardier insects, which may be tested at a later date for the presence of virus or used in tests of their ability to transmit virus through biting.

SUMMARY

Significant amounts of yellow fever virus have been recovered from the bodies of dead *Aedes aegypti* as long as 4 hours after death of the insect from exposure to ether, 17.5 hours after death from starvation, 20 hours after death from exposure to tobacco smoke, and 45.5 hours after death from exposure to potassium cyanide fumes.

On the contrary, following death from exposure to chloroform vapor, most of the virus activity was lost in 30 minutes and was totally absent in 1 hour.

Virus has been recovered from a significant number of lots of *Haemagogus equinus* and *Haemagogus spegazzinii* which died of "natural causes" during the course of laboratory transmission experiments.

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THE EFFECT OF PROLONGED STORAGE OF SERA ON YELLOW FEVER PROTECTION TESTS[1]

JOHN C. BUGHER

In studies such as those on the duration of immunity following yellow fever vaccination, it has been customary to use the results of the mouse protection test as a measure of the immune state; and in order to procure a time series, sera taken at various periods are compared in the same protection test run. This immediately raises the question of the effect of the storage on the older sera in the production of any differences of behavior between old and recent material.

There has been no satisfactory answer to this question, although a tentative approach to the problem has been made from time to time. Lloyd and Penna (1) compared the antibody content of desiccated and fresh immune serum over a period of nine months and concluded that there had been no demonstrable decline in antibodies in the desiccated material. These comparisons were made between desiccated sera from rhesus monkeys and fresh sera from the corresponding monkeys at the later periods. Fox and Cabral (2) conducted a somewhat more precise experiment in which desiccated and undesiccated sera from three immune rhesus monkeys were compared at intervals up to nine months, all sera being stored at 5°C. They found no difference between the two types of preparation and no indication of decrease in the amount of antibody. They therefore extrapolated their findings to four years, the period under consideration in their postvaccination study, and assumed that if there had been any change from storage, it was small.

On reopening the laboratory for yellow fever studies at Yaba, Nigeria, in the quarters occupied by the West African Yellow Fever Commission from 1924 to 1934, a large number of sera in ampoules were found in a packing case in one of the outbuildings along with the original protection test records of these same sera, which were part of those collected in a study of yellow fever immunity in West Africa during 1931–33. After testing, the remaining portions had been sealed in hard glass ampoules and stored at the ambient temperature. On the departure of the Commission, these sera were boxed, left in a small building covered with corrugated sheet steel, and subsequently forgotten. While the average annual temperature of Yaba is approximately 28°C. the sera must have endured considerably higher temperatures over these years, since during the daytime the hot tropical sun beat down upon the metal roof of the building[2] only a few feet over the boxes in which the ampoules had been left. Thus for slightly over 12 years, this material had been stored in almost the worst possible conditions, particularly with respect to temperature.

Fortunately, the records which were found with the abandoned sera were very complete, giving not only the results of the tests but a detailed description of the technique of each run. Since the same technique was not followed throughout the period of the original study, this was very important and made it possible to duplicate the original test procedure exactly.

From the stored material, sera originally protection-test positive were selected, together with a smaller number of inconclusives and negatives. From these were discarded all sera with evidence of hemolysis or old contamination, so that the specimens reserved for test were clear, free from hemolysis, and with less than one millimeter of sediment in the bottom of the ampoule. The remainder were then checked against the original protection test runs and where there was evidence of an unsatisfactory run or undue technical variation, sera tested in such runs were also excluded from further study. There remained 129 sera of good quality, tested originally under reasonably uniform conditions.

[1] The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of The Rockefeller Foundation.

[2] Recent observations in this building show that during most of the day the temperature exceeds 37°C., approaching 40°C. During the night there is a drop of several degrees. It appears that throughout the year the average temperature in this closed structure has not differed greatly from 37°C.

PROCEDURE

All sera were tested in standard intraperitoneal runs in mice of the Swiss strain, using the following technique:

The virus suspension was prepared by grinding in a porcelain mortar the brains of mice ill with neurotropic yellow fever virus. Grinding was continued for 15 minutes so that a smooth paste was formed. For each brain used there was added 1.5 cc. of 0.9 per cent salt solution to make a suspension approximately 20 per cent by weight. Smoothness of suspension was further insured by repeated aspiration of the material with a pipette. To 3.0 cc. of each serum, 1.5 cc. of the virus suspension was added and the tube was agitated thoroughly. On completion of the mixtures, including controls, the inoculation of mice was begun. Two hours previously the mice had received an intracerebral injection of 0.03 cc. of a 2 per cent solution of soluble starch under anesthesia. Six mice were injected intraperitoneally, without anesthesia, with each serum-virus mixture, the individual inocula being 0.6 cc. The time spent in inoculation varied between 40 minutes and one hour.

The virus strain used was that known as the French neorotropic strain established in mice by Theiler (3). The retests employed the first mouse passage of Theiler's Standard Virus 38 which itself is the 250th passage of the same strain.

The mice were observed daily through the tenth day, and the deaths were recorded. The final results of both the original tests and the retests were expressed as a fraction, called the survival ratio, of which the numerator is the number of survivors at the end of the tenth day and the denominator is the number of mice living on the fourth day (4).

The times of starching and inoculation used in the original tests were carefully observed in the retests. The mice used were in part descended from the original stock used in the old tests; those of the second stock show no difference in susceptibility to yellow fever virus when compared with the old Yaba strain. The ages of the mice were from 42 to 60 days, covering a span similar to that of the mice in the original tests.

RESULTS

The results of the tests are presented in the form of a frequency table in which the new test values are distributed with relation to the original survival ratios. Table 1 thus contains all of the relevant information of this study.

TABLE 1

Correlation of New Test Results with old Survival Ratios

	ORIGINAL	RETEST SURVIVAL RATIOS							
	S R	6/6	5/6	4/6	3/6	2/6	1/6	0/6	Total
	6/6	19	6	4	4	4	3	12	52
	5/6	8	5	4	4	4	5	12	42
	4/6	0	0	0	0	1	0	3	4
	3/6	0	0	1	1	2	1	6	11
	0/6	0	0	0	0	0	0	20	20
	Totals. ..	27	11	9	9	11	9	53	129
Normal human serum.....				0	0	0	2	6	8
Immune human serum pool 1:1.....		4	0	0	0	0	0	0	4
Immune human serum pool 1:10.....		4	0	0	0	0	0	0	4

It is evident that a marked shift to the lower survival ratios has occurred in the recent tests, and since every known factor of consequence has been maintained comparable in the two series of tests, it is reasonable to conclude that the shift is an effect due to storage of the sera. Further, it is clear that this effect is not a uniform one, for there tends to be a piling up of 0/6 results in excess of expectation. The same phenomenon is to be observed with sera that originally gave 5/6 survival ratios.

Table 2 has been computed from the formula:

$$P = (n+1)\, {}_nC_r\, {}_nC_s \cdot \frac{(r+s)!}{(2n-r-s+1)(2n-r-s+2)\cdots(2n+1)}$$

where P is the probability of the occurrence of the result, n is the number of mice in a group (in this case, 6), r is the original survival number, and s the number of survivors in the group whose probability P is desired (5). This table gives the expectations of survivals in a second sample when the first sample has shown the number specified (6). This implies all results "equiprobable" in the beginning, which cannot be assumed here. Still, table 1 may be assumed to show something like the expected distribution of first and second tests, if the potency of the sera had remained the same.

By comparing tables 1 and 2, it becomes more evident that the change toward less protective power was not a uniform one in all ampoules but instead was highly variable; so that instead of a unimodal curve, with a peak somewhere in the midrange for the sera originally giving 6/6 results, there are maxima at either extreme such as may be expected in a mixture of positive and completely negative sera as shown by Muench (7).

TABLE 2

Expected Retest Values of Sera of Table 1 Assuming No Change from Storage

Original	Retest survival ratios							
S R	6/6	5/6	4/6	3/6	2/6	1/6	0/6	Total
6/6	28	14	6	3	1	0	0	52
5/6	11	12	9	6	3	1	0	42
4/6	0	1	1	1	1	0	0	4
3/6	0	1	3	3	3	1	0	11
0/6	0	0	0	1	2	6	11	20
Totals....	39	28	19	14	10	8	11	129

In order to obtain some measure of the loss of antibody, an immune human serum pool was prepared from sera that had given 6/6 results in an intracerebral protection test of approximately the same sensitivity as the intraperitoneal test used for this study. These sera were from persons with naturally acquired immunity along the Cross River in Nigeria and compared favorably with the type of material originally tested in the yellow fever survey. This pool has been used as the immune serum control in the intraperitoneal retests and has given consistent 6/6 survival ratios.

An intraperitoneal antibody titration was performed with this pool using the same technique employed in the repeat tests. The results are shown in table 3.

Referring to the data of table 1, the group of 52 sera formerly giving 6/6 survivals or 100 per cent now gives only 58 per cent surviving mice; that is, of 312 mice inoculated with serum mixtures of this group, 183 survived. By interpolation, this corresponds to a dilution of 1:94 of the immune human serum pool IHSP-I. Taking this group of 52 old sera as a whole, it may be said that in 12 years the antibody content has been reduced to approximately 1 per cent of its original value. This figure given for the reduction of antibody is clearly only an indication of the general magnitude of the remaining activity and not a precise measure of it in any one serum, since there was a striking disparity of rate of change among the various ampoules.

TABLE 3

Run IP-6. Antibody Titration of Immune Human Serum Pool 1

Dilution	Survival ratio
1:16	12/12
1:64	8/12
1:256	2/12
1:1024	1/12

DISCUSSION

While a large amount of antibody was lost in this material in the 12 years of storage under adverse conditions, the extent to which antibody could still be demonstrated is striking, although the test has a low order of sensitivity.

It is especially noteworthy that there were no instances of previously negative sera giving positive results after the long storage, a point that often causes concern in testing old material. While the evidence is clear in this instance, where hard glass ampoules of high quality were used, it is not safe to generalize concerning the inocuousness of more soluble glasses, such as are frequently employed. More studies should be made before concluding that false positives are never produced by solution of glass into the contained serum.

Fox and Cabral (2) noted that sera stored four years gave less protection than fresh sera of the same persons examined in the same runs, when the standard intraperitoneal protection test was used. Since they had reason to believe that yellow fever

antibodies do not increase spontaneously with time, they adopted another form of test in which this difference did not appear. A good illustration of the behavior of the four-year sera in the standard test is shown in figure 5 of their paper. For comparison, the data of the study of the 12-year sera have been thrown into the same graphic form in figure 1. The original 0/6 sera have been omitted since we are concerned with the effect of storage on antibody demonstration. It is at once clear that the observation made by Fox and Cabral with the standard test is fully accounted for by

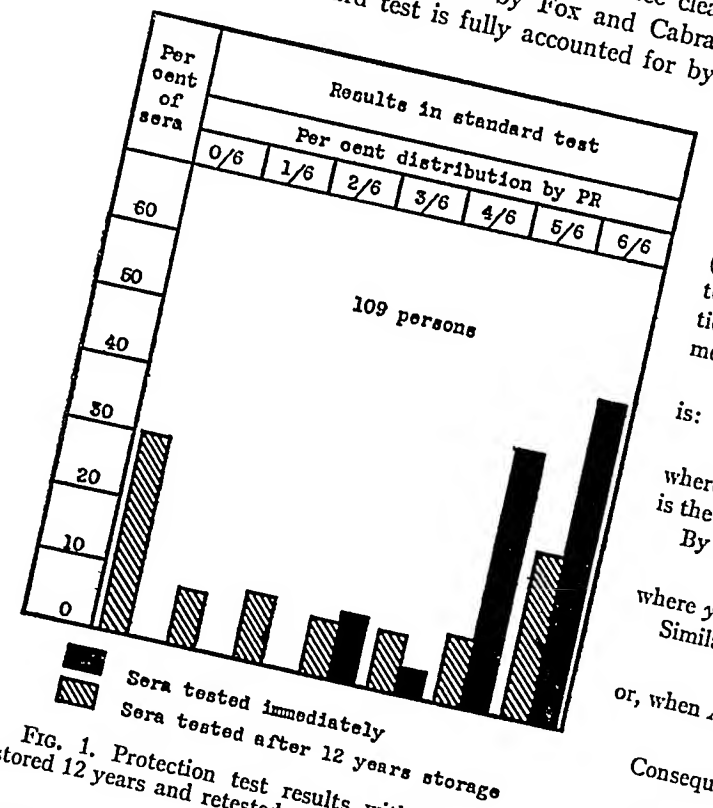

FIG. 1. Protection test results with human sera stored 12 years and retested using original technique.

storage alone even when allowance is made for the much more favourable temperature conditions.

It is informative to consider these yellow fever data in relation to observations on the deterioration of diphtheria antitoxin.

Anderson (8) reported his findings at 5°C. and 15°C. to which MacConkey (9) added a measurement at 36°C.:

TEMPERATURE OF STORAGE	LOSS OF PROTECTIVE POWER IN ONE YEAR
degree C.	*Per cent*
5	6
15	10
36	50

Glenny (10), in a careful study of two lots of antitoxin stored in sealed glass ampoules at 37°C., measured both the L_o and L_+ quantities of the stored material. He found a marked diminution in the local reaction, upon which the L_o is based, while the lethal effect was relatively well sustained. No true F_o value remained at the longer periods. Consequently, Ehrlich's "differential region" no longer existed.

Glenny's results, expressed in L_+ units per cubic centimeter were as follows:

LOT	TIME IN YEARS			
	0	4.5	6 75	7
E 148	440	85	40	40
469	470	65	55	

Taking MacConkey's and Glenny's data together (using the 36° and 37° observations as of the same temperature level), it is possible to gain some information as to the order of the reaction. The basic statement for the first order reaction (11) where

$$A \rightarrow B + C + \cdots$$

is:

$$dy/dt = -k_1 y,$$

where y is the amount of antibody at time t and k_1 is the velocity constant of the reaction.

By integration,

$$y = y_o e^{-k_1 t}$$

where y_o is the original amount of antibody.

Similarly, for the second order reaction,

$$A + B \rightarrow C + D + \cdots$$

or, when A and B are equal,

$$2A \rightarrow C + D + \cdots$$

Consequently,

$$dy/dt = k_2 y^2$$

Whence,

$$y = \frac{Y_0}{1 + k_2 y_0 t}$$

where the symbols have the same significance as before save for the constant.

From Glenny's observations at 6.75 years, the mean value for k_1 is 0.336 and for k_2 it is 1.299. Using these values of the constants and the equations given above for the first and second order reactions, table 4 has been computed.

From table 4, it is evident that the reaction of the second order fits the observed data within the limits of accuracy of the method used.

The reaction velocity constant is related to temperature as follows:

$$\log k = C - \frac{a}{T}$$

where C and a are constants and $\frac{1}{T}$ is the absolute temperature (11). Plotting $\log k$ against T should

therefore give approximately a straight line. The quantities concerned are shown in table 5 and plotted in fig. 2.

TABLE 4

Deterioration of Diphtheria Antitoxin Stored at 36°–37°C.

Combined Data of MacConkey and Glenny*

TIME	AMOUNT ANTITOXIN EXPECTED		AMOUNT ANTITOXIN OBSERVED
	$k_1 = 0.336$	$k_2 = 1.229$	
years			
0	1.00	1.00	1.00
0.5	0.84	0.61	0.64
1.0	0.71	0.44	0.50
4.5	0.22	0.17	0.17
6.75	0.10	0.10	0.10
12	0.02	0.06	

* Initial value defined as unity.

Since in 12 years the reduction in the amount of yellow fever antibody was of the order of that expected with diphtheria antitoxin kept under similar conditions, it seems reasonable to assume, lacking more information, that the order of the reaction and the temperature relationships are closely similar in the two systems. Under such an assumption, k_2 would be 0.055 at 5°C. and the loss in four years would be 18 per cent.

However, while it is thought that the general

TABLE 5

Deterioration of Diphtheria Antitoxin

Velocity Constant k_2 at Various Temperatures

AUTHOR	TEMPERATURE		TIME	k_2	$\log_{10} k_2$
	°C.	Å			
			years		
Anderson.....	5	278	1	0.064	−1.194
Anderson.....	15	288	1	0.111	−0.955
MacConkey...	36	309	1	1.000	0
Glenny.....	37	310	4.5	1.159	0.063
Glenny.....	37	310	6.75	1.128	0.052
Glenny.....	37	310	7	1.428	0.154

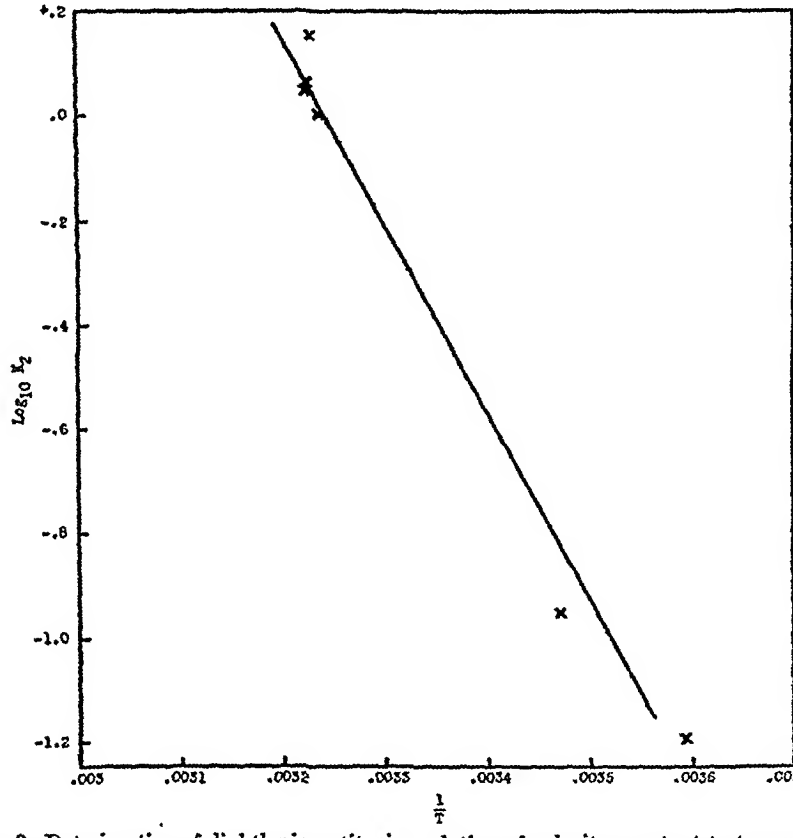

FIG. 2. Deterioration of diphtheria antitoxin: relation of velocity constant to temperature

For practical purposes, there are only three points to be fitted. The line which has been drawn to represent the temperature effect on the velocity constant is:

$$\log_{10} k_2 = 11.566 - \frac{3570}{T}$$

results of the study of the 12 year sera are consistent with this interpretation, it is also clear that individual ampoules depart widely from this and show a much greater decline. The piling up of 0/6 survival ratios suggests that other factors were also operating to accelerate the diminution of antibody, so that an appreciable fraction have lost all evidence of antibody content while another large proportion have retained it quite well, and there is not the preponderance of intermediate survival ratios which one would expect from a uniform decline with time.

It is impossible to enumerate the factors responsible for this variation in rate of loss, but it is clear that one cannot assume uniformity of change in sera stored for several years, even under the best of conditions. Further, it may be suggested that the reduction with time of the correlation between various methods of measuring antibody found by Glenny with diphtheria antitoxin may hold likewise for yellow fever antibody. In such case, the improvement in results noted by Fox and Cabral when their stored sera were compared with fresh ones by the use of the young mouse test, may have been due not so much to the greater sensitivity of the test as to its being different from the standard test and responding to a slightly different characteristic. The fact that the difference noted with the standard test could be eliminated by the young mouse test, and indeed inverted to some extent, serves to emphasize that the various forms of the protection test are not identical in minor respects.

CONCLUSIONS

1. Yellow fever immune sera after 12 years of storage in glass ampoules under tropical conditions showed a marked quantitative loss of antibody, as shown by the standard intraperitoneal protection test.

2. Previously negative sera, stored under the same conditions, gave only negative results.

3. Under these conditions, it appears that the average loss of protective power by an immune serum may be of the order of 50 per cent per year. Marked individual variations may occur, however, so that the deterioration may be greatly accelerated in individual instances.

4. Yellow fever antibody appears to deteriorate at a velocity similar to that of diphtheria antitoxin, some characteristics of which are computed.

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RELAPSING FEVER IN DENTON COUNTY, TEXAS

Report on Finding the Tick, Ornithodorus Turicata, Naturally Infected

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Relapsing fever has been reported from many parts of Texas in the past several years and a number of caves have been cited as harboring ticks both infected and non-infected with the spirochete of relapsing fever (Graham, 1931; Kemp et al, 1935; Coleman and Wright, 1942). In a discussion note following Graham's paper, Dr. T. C. Terrell of Ft. Worth reported a series of replapsing fever infections studied by him originating from a Sam Bass Cave in Denton County. In the reports of Kemp and his associates (1934 and 1942) mention was made of a cave in Denton County, Texas, also called by them the Sam Bass Cave. In none of these reports was the exact location of the cave given. The one studied by Kemp, Moursund and Wright over a period of several years was reported to be infested with the tick *Ornithodorus turicata*. During the period of their study they did not find these ticks to harbor the relapsing fever spirochete though frequent collections were made. No authentic human infections of relapsing fever have been traced to this cave since 1931. Fisher (1944) stated that though the ticks he collected there did not harbor the spirochetes as a natural infection they could acquire it by feeding on rats infected with his laboratory strain.

Report of Naturally Infected Ticks in Denton County

In search for a source of the tick, *Ornithodorus turicata*, the author was directed to a cave in the south central portion of Denton County, Texas, near the town of Roanoke. It proved to be rather small and near the summit of a hill called by some of the natives "Jinglebob Hill". (Its location is shown on the map in figure 1.)

Rocky ledges overhanging the cave form a point from which one can view the adjacent valley through which Denton Creek flows. Apparently this spot is quite well known locally for trails leading to the point and recent inscriptions on the rocks were observed. The cave is called Carpenter's Cave by some of the natives in that region. Figure 2 is a photograph of the cave entrance.

Three trips have been made by the author to this cave in the past year and each time ticks were collected from the sandy floor in its more remote portions. Droppings and tracks indicated that rodents frequented the cave. It was found that the ticks were most numerous in dry areas of the cave floor where animal droppings were more frequent and ticks were not found in areas where the sand was quite moist from recent rains.

The ticks were identified as *Ornithodorus turicata* by Dr. Brennan of the United States Public Health Service. Both nymphal stages and adults were collected, but no hexapod larvae were found. Though most of the ticks apparently had not fed for some time, a few showed evidence of a relatively recent meal.

Groups of four ticks each were fed upon white rats which were subsequently observed by means of thick blood smears for evidence of infection with the relapsing fever spirochete. Ticks from each of the three collections were found to be infective for rats in which the course of the infection showed the typical relapsing phenomenon. No studies on the percentage of ticks infected were made.

Two members of the first collecting party found ticks upon their bodies, at least one being attached in each case. Seven days after having been bitten one member developed a typical case of relapsing fever. The spirochetes were found in thick blood smears and two rats became positive seven and ten days after inoculation with his blood. The infection was terminated with neoarsphenamine and no relapses have occurred.

Nine ticks from the cave were allowed to feed upon a congenital luetic child, age 13. Five days later he became ill rather suddenly with chills and a rapid rise in temperature. Spirochetes were demonstrated in his blood by means of thick smears and animal inoculations. Four relapses occurred at intervals of 3 to 5 days. Spirochetes

FIG. 1. Map showing location of cave
(Adapted from State Highway Department map of Denton County)

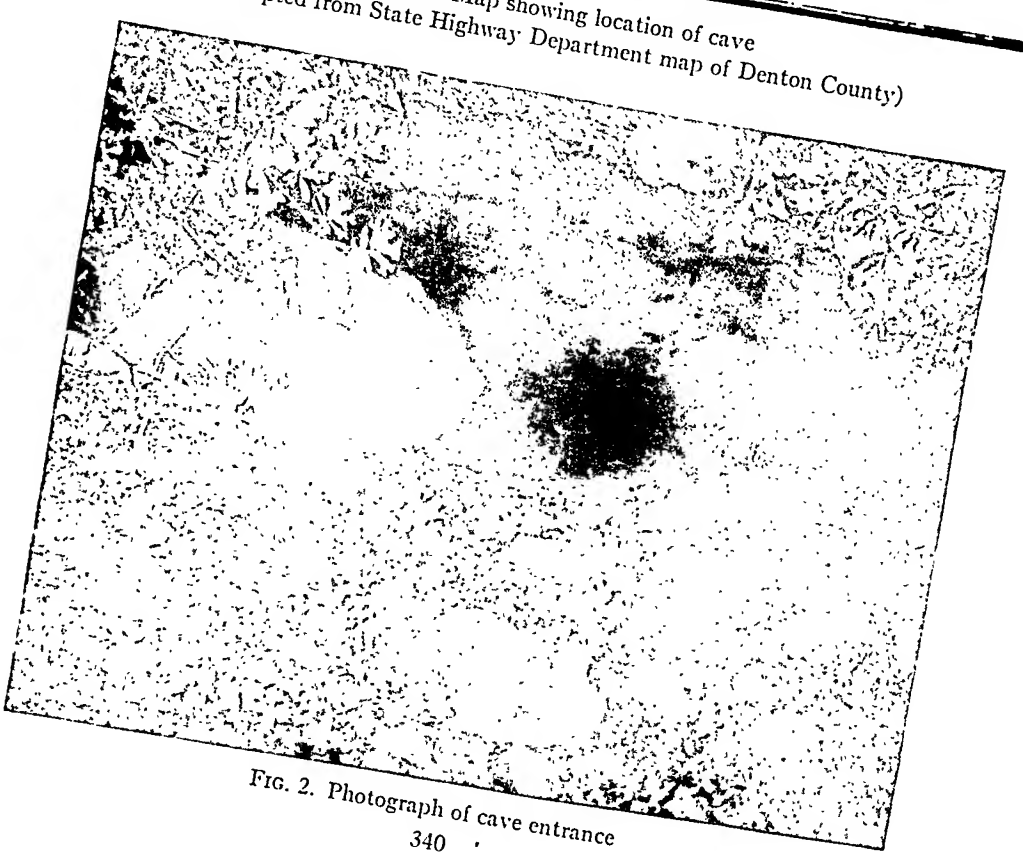

FIG. 2. Photograph of cave entrance

were demonstrable in his blood during each paroxysm.

DISCUSSION

Following the experimental evidence of natural infection with relapsing fever spirochetes in ticks collected from the cave under investigation as indicated by the animal and human infections, an attempt was made to identify this cave with the one studied by Kemp et al in this region. On one of the collecting trips Mr. Waters of the Medical Art Department without direction led the author to the cave herein reported and identified it as one which he had visited with Kemp several years ago. From a description sent him, Kemp has since identified it as the Sam Bass Cave of his earlier studies (Kemp et al, 1934 and 1942).

It will be recalled that in 1930 and 1931 infections of relapsing fever were attributed to this cave, the most notable of these being that of Mr. W. G. Bruce, United States Department of Agriculture, Bureau of Entomology, which was reported by Kemp, Moursund, and Wright (1934). In the years following these infections Kemp and his associates (1934 and 1942) repeatedly collected ticks from this locality but found none infected. Then in 1944 the author in collections made over a period of several months found experimental evidence of natural infection.

One of two conditions could explain the failure of Kempt et al to find infected ticks:

(1) Following 1931 the infection disappeared completely from the cave. One would expect the proportion of infected individuals in a tick population depending solely on "hereditary" transmission to decrease and perhaps finally disappear since it is probable that only a fraction of the progeny of an infected female tick are infected through the ovum (Francis, 1932; Brumpt, 1933; Kemp et al, 1934; Herms and Wheeler, 1936).

(2) There were so few infected individuals present during the studies of Kemp et al that even repeated collections failed to demonstrate them (as suggested by Bohls, 1942).

A variety of animals captured in or near caves or burrows harboring ticks of the genus *Ornithodorus* have been found naturally infected with relapsing fever spirochetes (Bohls, 1942). Such animals could play a rôle in the re-introduction or enrichment of the infection in one of several ways:

(1) Infected ticks carried into the cave on animals from some other source may have detached themselves there. The short feeding period of the tick is not in favor of this method.

(2) Animals infected in another focus might have visited the cave while the infection was still active, thereby infecting ticks which may have fed on them.

(3) In the event that the infection did not die out completely, susceptible animals inhabiting the cave may have become infected and resided long enough to allow many of the ticks to become infected. Such enrichment could markedly increase the proportion of infected ticks in a relatively short period of time.

Specific experimental proof of any of these possibilities has not yet been obtained.

SUMMARY

1. A cave harboring the tick *Ornithodorus turicata*, is reported from Denton County, Texas.

2. The cave was identified as the Sam Bass Cave studied previously by Kemp and his associates.

3. For the first time experimental evidence is presented that many of these ticks are naturally infected with the spirochete of relapsing fever and that they are able to transmit the infection to man and white rats.

4. An attempt has been made to indicate the possible mechanisms through which the infection in this area apparently disappeared and reappeared.

ACKNOWLEDGMENTS

The author is indebted to Dr. MacDonald Fulton, Department of Bacteriology, Southwestern Medical College, Dallas, Texas, for his advice and guidance throughout this study; to Mr. Lewis Waters, Department of Medical Art, Southwestern Medical College, for his aid in identifying the cave and for performing the photographic work involved; to Dr. James M. Brennan, Entomologist, U. S. Public Health Service, Rocky Mountain Laboratory, Hamilton, Montana, for the identification of the ticks; to Dr. G. T. Caldwell, Dr. J. M. Hill and Dr. Sol Haberman, Department of Pathology, for their criticisms of the manuscript.

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EARLY RESULTS OF THE TREATMENT OF AFRICAN TRYPANOSOMIASIS WITH TWO NEW ARSENICAL PREPARATIONS

(Melarsen Oxide and 70A)[1] Preliminary Report

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The current treatment of African trypanosomiasis with antrypol or Bayer 205 and tryparsamide is usually effective. However, it is not ideal, and improved chemotherapeutic agents of reduced toxicity and higher effectiveness and greater ease of administration are desirable. It was with this in view that the present study of the effect of two new drugs on African sleeping sickness was undertaken.

These investigations were carried on in Liberia, West Africa, under the auspices of the American Foundation for Tropical Medicine, Inc. Through the courtesy of the Firestone Plantations Company, the hospital of the Firestone rubber plantation at Harbel, Liberia, was made available. Our patients were drawn from the native plantation laborers. These infected laborers came from different parts of Liberia where apparently the disease is established, but during the period that they remained on the Plantation proper, we consider that they were free from the risk of re-infection. This report deals with 26 such patients: 16 were treated with Melarsen oxide alone, 8 with 70A alone, and 2 with 70A followed by Melarsen oxide.

Melarsen oxide is a trivalent arsenic oxide derived from the pentavalent compound Melarsen previously introduced into therapy by E. A. H. Friedheim. Melarsen oxide contains 22.8 per cent trivalent arsenic, is very slightly soluble in cold water, but will form 5 per cent solutions in pure propylene glycol. It appears to be very stable, thus no decomposition of the solid chemical was noted in 2½ years, nor of the propylene glycol solutions in 10 months. Preliminary animal studies, performed by others, indicated marked *in vivo* trypanocidal action, a wide spread between curative and toxic doses and effectiveness after oral administration. Parke, Davis and Company very kindly made available a supply of this compound for clinical trial.

Melarsen oxide was given orally to 12 patients at a dose of 3 milligrams per kilogram for 5 to 8 consecutive days. Eight of these cases had signs or symptoms of neurological involvement; the spinal fluid cell counts ranged from 65 to over 1200 per cubic millimeter with an average of 357 cells; 5 also had trypanosomes in the spinal fluid. After a single course of treatment all individual counts were reduced, the range being from 7 to 556 and the average falling to 138; 3 of the 5 with trypanosomes had become negative. The 4 patients with most abnormal fluids were given a second course which reduced the cell counts from an average of 268 to 90; both patients who had shown trypanosomes in the spinal fluid became negative. Four months after cessation of treatment, one of these patients who had received two courses had a higher spinal fluid cell count than the previous month, indicating a relapse.

Oral Melarsen oxide was also administered to four patients with normal spinal fluid cell counts in the early stage of infection. In all, the lymph node punctures and thick drops of blood had become negative at the end of a single course. No relapses were noted during two months after treatment, the maximum period of time we have as yet been able to follow a patient. Some of our neurological cases also had positive bloods or lymph nodes. These became negative after one or two courses of Melarsen oxide and have remained so for periods up to 3½ months.

Intravenous Melarsen oxide was given to 8 patients at a dose of 0.1 mg./kg. daily for 7 consecutive days. Five cases were blood or lymph node positive only; these became negative after the first course of treatment and have remained so for periods up to 2 months. Three other cases were of the neurological type and had previously been treated by other drugs without success. One was clearly benefited by Melarsen oxide, the cell

[1] Read at the Fortieth Annual Meeting of the American Society of Tropical Medicine, at St. Louis, Mo., November 13–16, 1944.

count falling from 110 to 9 cells; the other two did not respond; we hope to determine whether this may be attributed to arsenic resistance or to inefficacity of the drug.

The second new drug employed, called 70A, is a phenylarsenoxide in which the arsenic is also trivalent. We are indebted to Dr. Harry Eagle for providing us with our supply.

70A was used on 10 patients. After trials for sensitivity with one or two half doses, 70A was then given 40 mg. per injection, the injections being given thrice weekly and the total dose amounting to between 360 and 400 mg. In all of 7 early cases with no neurological involvement, the blood and lymph nodes became negative after one course, remaining so up to 5 months, the maximum period any patient was under observation. With this compound, a rapid reduction in size of the cervical and epitrochlear lymph nodes was frequent and remarkable. Thus, 70A appeared to be effective in the early stages. However, this compound seemed to have little or no action on the spinal fluid in meningo-encephalitic cases. Thus two of our patients had a much higher spinal fluid cell count after treatment than before, and in one of the patients, trypanosomes, previously absent, were found in the spinal fluid very shortly after the patient had received 380 mg. of 70A.

To summarize our results, Melarsen oxide has proved to be effective in both of the clinical stages of sleeping sickness. With doses which accomplished this, no toxic reactions were observed. We believe these results are sufficient to warrant further attentive study of the compound, particularly to determine the optimum dosage and to compare its efficacity, toxicity, and relapse rate with drugs now in use. As to the comparative efficacy of the oral and intravenous methods of administration, the parenteral route is to be preferred for maximal effect. However, the drug given *per os* is undoubtedly effective and this method may be useful as a second choice, particularly before the nervous system is involved. In view of the indisputable efficacy of Melarsen oxide in meningo-encephalitic cases, it is interesting to note that arsenic determinations, performed by others on experimental animals, indicate that brain concentrations of Melarsen oxide are about the same as those of the heart and muscle tissue, and ran from two to three times as much as the blood concentration at the same time. Liver and spleen contained five to ten times as much, and kidneys thirty times as much as the brain.

In regard to 70A, it seems clear that at the doses employed, 70A was effective in the early stage but neither prevented nor ameliorated cerebral involvement.

AN INQUIRY INTO THE GROWTH FACTOR OR FACTORS OF CERTAIN BLOOD AND TISSUE FLAGELLATES[1]

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INTRODUCTION

Neither Ray (1932) nor Berrebi (1936) was able to grow the leishmanias in blood serum or in washed red blood cells. Using a slight modification of Kligler's medium (1925), Adler (1934) cultured these organisms in the presence of serum which contained only traces of whole blood (1:5,000). Lwoff (1939) claimed that there are three factors essential for the growth and multiplication of the leishmanias and of *Trypanosoma cruzi*. Two of these factors were supposedly identified as ascorbic acid and hematin, while the third was an unknown fraction in the blood serum. Senekjie (1939, 1941, 1943) cultured the leishmanias in a liquid medium which contained rabbit serum free of hemoglobin. He likewise grew the leishmanias, as well as *Trypanosoma cruzi*, in egg-liver extract medium which was free of ascorbic acid and hemoglobin. By serial subcultures the leishmanias survived in egg-liver extract medium for four to six generations and *T. cruzi* for eight to ten generations. The first few generations were very rich, but progressively the growth became less and less abundant, until it ceased. When such a scanty culture was transferred to a blood medium, the organisms again grew very abundantly. Apparently they had stored some vital factor, and the reduced growth and multiplication were due to a progressive consumption of this stored factor. The present writers have observed that liver extract solution is not essential and that egg medium overlayed with physiological salt solution or Locke's solution gives comparable results (unpublished data). Thiamin chloride, nicotinic acid and pyridoxin have temporary growth-stimulating properties, so that in leishmania broth with these vitamins the organisms grow and multiply for more than three weeks with or without subculturing. Ascorbic acid has no such stimulating action while riboflavin and calcium pantothenate retard growth.

The object of this study has been to determine the properties of the factor or factors responsible for the growth of the leishmanias and *T. cruzi* in culture.

MATERIALS AND METHODS

Leishmania agar rabbit serum medium. Five per cent normal rabbit serum was added to liquid leishmania agar (Senekjie, 1943) at 45°C. The medium was then put into test tubes and made into slants. This culture medium constituted the control on which the organisms were known to thrive. Some batches of this medium were heated at 70°C. and 100°C. in a water bath for 30 minutes and then cooled as slants.

Leishmania agar washed rabbit erythrocyte medium. Defibrinated erythrocytes of normal rabbits were washed four times in sterile physiological salt solution in an attempt to remove all traces of serum. These washed erythrocytes were suspended in enough physiological salt solution to make up the original blood volume, and this suspension was then added to melted leishmania agar at 45°C. to make 10 per cent of the medium. The medium was then tubed and made into slants.

Leishmania agar egg white medium. Fresh sterile egg white was added to liquid leishmania agar at 45°C. to give final concentrations of 5 and 10 per cent albumin in the medium, which was

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Read by Title at the Fortieth Annual Meeting of the American Society of Tropical Medicine, at St. Louis, Mo., November 13–16, 1944.

then made into slants. Some batches of the medium were heated in a water bath at 70°C. and 100°C. for 30 minutes. Twenty-five hundredths per cent para-aminobenzoic acid in physiological salt solution and 0.1 per cent thiamin, 0.1 per cent nicotinic acid, 0.1 per cent pyridoxin each in physiological salt solution; as well as equal amounts of 0.1 per cent nicotinic acid plus 0.1 per cent pyridoxin in physiological salt solution, and 0.25 per cent para-aminobenzoic acid plus 0.1 per cent nicotinic acid plus 0.1 per cent pyridoxin in physiological salt solution, were used as overlaying solutions. Thiamin was sterilized by filtering through a Seitz filter, while the other vitamins were autoclaved.

Leishamania agar hemoglobin medium. One gram of Bacto beef hemoglobin (Difco) was dissolved by gentle heating in enough normal NaOH (approximately 20 cc.) to get the hemoglobin into solution. The solution was then neutralized with normal HCl, autoclaved, then added to liquid leishmania agar at 45°C. to give a final concentration of one per cent, and then made into slants.

Rabbit hemoglobin medium was prepared by centrifuging 7 cc. of defribinated rabbit's blood (containing approximately one gram of hemoglobin), decanting the serum and washing the erthrocytes 4 times with sterile physiological salt solution, in an attempt to remove all traces of serum. The washed red blood cells were hemolized with normal NaOH, and enough of the base was added to make the material fluid (approximately 20 cc.). Sufficient normal HCl was added to neutralize the solution which was autoclaved, then added to 100 cc. of liquid leishmania agar at 45°C. and made into slants. Human and sheep hemoglobin were prepared in a similar manner except that citrated blood was used in place of defibrinated blood. Tubes of hemoglobin leishmania agar media were overlayed with 0.25 per cent para-aminobenzoic acid in physiological salt solution, and with 0.1 per cent thiamin, nicotinic acid, and pyridoxin each in physiological salt solution, as well as equal amounts of 0.1 per cent nicotinic acid plus 0.1 per cent pyridoxin in physiological salt solution, and 0.1 per cent nicotinic acid plus 0.1 per cent pyridoxin plus 0.25 per cent para-aminobenzoic acid in physiological salt solution.

Leishmania blood phenol agar. Concentrated liquid phenol was added to the liquid leishmania blood agar at 45°C. in the proportion of 1:1000, and the medium was slanted in tubes.

Leishmania albumin, leishmania euglobulin and leishmania pseudoglobulin agar. Rabbit serum albumin, euglobulin and pseudoglobulin were prepared by Dr. H. M. Powell, Biological Division, Eli Lilly Research Laboratories, in the following way.

"Nine hundred and twenty-five cc. of normal rabbit serum was taken from healthy rabbits on April 19, 1944. An equal volume of distilled water was added. To this combined volume of 1850 cc. was added 793 cc. saturated ammonium sulfate, making a 30 per cent saturated solution. This solution stood overnight at room temperature. Filtration was conducted on hard filter paper, and the precipitate was pressed and dialyzed free of ammonium sulfate in the cold through cellophane. This was considered the eugobulin fraction and was labeled B-7600A.

"The filtrate which had pressed through the hard filter paper measured 2540 cc. To this was added 1000 cc. saturated ammonium sulfate to attain a concentration of 50 per cent saturation. After standing, this solution was filtered through hard paper, and the precipitate was pressed and dialyzed as before. This solution was considered as pseudoglobulin and was labeled B-7600B.

"The above filtrate from the pseudoglobulin was fully saturated with the ammonium sulfate by adding the dry salt. The albumin which precipitated was filtered off through hard paper and pressed and dialyzed and given the number B-7600C.

"All three preparations, namely euglobulin, pseudoglobulin and albumin, proved reasonably free of sulfate, and each preparation was Seitz filtered to attain sterility. At no stage of the entire process was any heat, preservative, or other deleterious substance used. All of these solutions represented, of course, various concentrations of the separated proteins, due to the succeeding periods of dialysis becoming of necessity somewhat longer to free the material of sulfate and thereby increasing the volume of the dialyzed solutions." Albumin, euglobulin and pseudoglobulin were added to batches of the liquid leishmania agar at 45°C. to make ten per cent of the medium. Each mixture was then tubed and slanted.

Similarly, media were prepared containing 5 per cent euglobulin plus 5 per cent pseudoglobulin, 5 per cent euglobulin plus 5 per cent albumin, 5 per cent albumin plus 5 per cent pseudoglobulin, and 5 per cent each of albumin, euglobulin and pseudoglobulin incorporated into leishmania agar.

Five per cent solutions of the three protein fractions of rabbit serum in physiological salt

solution, singly and in combination, were prepared under sterile conditions to be used as liquid overlays on leishmania agar.

All of the above-mentioned media were inoculated with cultures of *L. donovani*, *L. brasiliensis*, *L. tropica* and *T. cruzi*, and subcultures were made on the same media to determine the time of survival. When no growth was observed by direct microscopic examination, subcultures were made on regular leishmania blood agar medium.

RESULTS

The three leishmanias and *T. cruzi* were first cultured on leishmania agar rabbit serum medium, and on the same medium which had been preheated at 70°C. and 100°C. for 30 minutes. Physiological salt solution was used as an overlay on all of the above media. In unheated serum medium, the growth was very abundant as it was on the regular leishmania blood agar medium. On this medium preheated at 70°C., the growth was normal for one to five cultures, then became poor, but later it was normal and abundant. On the medium preheated at 100°C., there was a progressive diminution in growth: *L. tropica* died in the first culture tube, *L. brasiliensis* ceased growing after the first subculture, while *L. donovani* and *T. cruzi* were slightly more tolerant and survived through the fourth and third subcultures respectively. Abundant growth was obtained in the unheated controls.

The same four organisms were then cultured on leishmania agar washed rabbit erythrocyte medium, with physiological saline overlay. *L. tropica* grew only in the first culture tube, *L. brasiliensis* stopped growing after the first subculture while *L. donovani* and *T. cruzi* ceased growing after the fourth subculture. It was again apparent that the time of survival of *L. donovani* and *T. cruzi* is longer than that of *L. tropica* and *L. brasiliensis*. The controls showed luxuriant growth.

L. tropica and *T. cruzi* were likewise cultured on unheated leishmania agar egg white medium on this medium preheated at 70°C., and at 100°C. for 30 minutes. Each of the above media had physiological salt solution as an overlay. These two organisms were also cultured on unheated leishmania agar egg white medium overlayed separately with 0.25 per cent para-aminobenzoic acid, 0.1 per cent thiamin, 0.1 per cent nicotinic acid, 0.1 per cent pyridoxin, 0.1 per cent nicotinic acid plus 0.1 per cent pyridoxin, and equal amounts of 0.25 per cent para-aminobenzoic acid, 0.1 per cent nicotinic acid, plus 0.1 per cent pyridoxin, each in physiological salt solution. On unheated leishmania agar egg white medium with physiological salt solution overlay, *L. tropica* survived two subcultures, while *T. cruzi* survived three. On leishmania agar egg white media preheated at 70°C. and 100°C., with the same overlay, *L. tropica* survived one subculture, while *T. cruzi* survived three. Neither para-aminobenzoic acid nor any of the vitamin B complexes tested were found to contain the growth-promoting factor or factors, since the organisms never survived more than one or two subcultures. The unheated controls showed abundant growth.

L. tropica was cultured on leishmania agar beef, rabbit, human and sheep hemoglobin media and on these media overlayed with physiological salt solution, 0.1 per cent vitamin B fractions as well as para-aminobenzoic acid in physiological salt solution, singly and in various combinations. On rabbit and on human hemoglobin media overlayed with physiological salt solution, *L. tropica* did not grow, but on beef and on sheep hemogobin with the same overlay it grew through one subculture. Similarly, 0.1 per cent thiamin, 0.1 per cent nicotinic acid, 0.1 per cent pyridoxin and 0.25 per cent para-aminobenzoic acid and combinations of these solutions, when utilized as overlays, were shown to lack the growth-promoting factors. Controls all showed abundant growth.

T. cruzi was cultured on various hemoglobin media overlayed with physiological salt solution and with 0.1 per cent vitamin B fractions as well as 0.25 per cent para-aminobenzoic acid, singly and in combination. On leishmania agar human hemoglobin medium with physiological salt solution overlay *T. cruzi* survived only one culture, on sheep hemoglobin two, on beef hemogobin four, and on rabbit hemoglobin five. Similarly, on hemoglobin and vitamin combinations the organisms survived four to six cultures, but in all the tubes growth ceased on or before the sixth generation.

On 0.1 per cent leishmania phenol blood agar with physiological salt solution overlay, *L. tropica*, *L. donovani* and *T. cruzi* did not survive the first culture tube. *L. brasiliensis* was not utilized in this experiment.

Six-day-old cultures of *L. brasiliensis*, *L. donovani*, *L. tropica* and *T. cruzi* which were growing luxuriantly on leishmania blood agar medium were inoculated with *Penicillium notatum*. *L. donovani* and *L. tropica* died in 72 hours while *L. brasiliensis* and *T. cruzi* lived for 7 days.

All four flagellate organisms were cultured each

on leishmania albumin, euglobulin and pseudoglobulin media, with physiological salt solution utilized as an overlay, and on 5 per cent of the corresponding fractions in physiological salt solution as overlays on leishmania agar. The control consisted of leishmania agar overlayed with physiological salt solution. On the leishmania albumin medium the leishmanias survived two generations while *T. cruzi* survived three. On the leishmania euglobulin medium *L. brasiliensis* and *L. donovani* did not grow, while *L. tropica* and *T. cruzi* grew on the original culture tube but did not survive transfer. On the leishmania pseudoglobulin medium *L. brasiliensis* survived one culture, *L. tropica* two, *L. donovani* three, and *T. cruzi* four. In protein fractions used as overlays the survival rate was slightly less than when the fractions were incorporated into the medium.

Finally, these organisms were cultured on combinations of albumin, euglobulin, and pseudoglobulin incorporated into the medium. On the leishmania euglobulin-pseudoglobulin medium, *L. tropica* failed to grow, *L. brasiliensis* and *L. donovani* grew through two cultures, while *T. cruzi* grew through four cultures. On the leishmania euglobulin-albumin medium *L. brasiliensis* and *T. cruzi* grew through one culture, while *L. donovani* and *L. tropica* grew through two cultures. On the leishmania albumin-pseudoglobulin medium *L. tropica* grew through one culture, *L. brasiliensis* through two and *L. donovani* and *T. cruzi* both grew through three cultures. On the leishmania albumin-euglobulin-pseudoglobulin medium *L. brasiliensis* and *L. donovani* grew on the original culture but did not survive transfer, *L. tropica* survived one transfer and *T. cruzi* two transfers.

DISCUSSION

Experimental evidence during this investigation has shown that the growth-promoting factors are not present in erythrocytes washed free of serum; nor are they in the hemoglobulin of sheep, rabbit, man or beef, but they are present in blood serum. These factors are at least partially thermostabile at 70°C. for 30 minutes, but are destroyed at 100°C. for 30 minutes. The present study has demonstrated that these factors are not present in the albumin, euglobulin, or pseudoglobulin fractions of rabbit serum, or in combinations of these component protein fractions. The addition of various vitamin B complexes and para-aminobenzoic acid to the hemoglobin does not restore the growth-promoting effect of serum. Moreover, the factor or factors are not present in egg white. Further studies are necessary to identify the factor or factors concerned which may have been lost during dialysis of the serum protein fractions investigated.

An attempt was made to induce smooth to rough variation by growing the organisms in a phenolized medium, but in such a medium the organisms died.

Penicillium notatum killed cultures of the organisms studied in 3 to 7 days.

All of the data on the culture requirements of these organisms show that hemoglobin is not necessary for their growth, hence the term "hemo flagellates" as applied to them by certain workers is inappropriate.

SUMMARY AND CONCLUSIONS

1. The growth-promoting factor or factors for the blood and tissue flagellates studied are present in serum, but are not directly associated with albumin, euglobulin, pseudoglobulin or combinations of these three important fractions.

2. Neither hemoglobin nor washed erythrocytes contain the necessary stimulant.

3. This stimulant partially survives heating at 70°C. for 30 minutes, but is destroyed at 100°C.

4. The factor or factors are not para-aminobenzoic acid, pyridoxin, nicotinic acid, thiamin nor any other vitamins tested.

5. The essential stimulant is dialysable, and is probably lost during the process of fractionating the serum proteins.

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RODENTS, RABBITS AND TULAREMIA IN NORTH AMERICA: SOME ZOOLOGICAL AND EPIDEMIOLOGICAL CONSIDERATIONS[1]

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The term "rodents" is often used to include all small mammals, particularly those of an injurious nature, yet, properly speaking, the rodents, Order Rodentia, are a definite zoological group best characterized by the chisel-like incisor teeth, four in number, and the absence of canine teeth. The rabbits, which were previously classed as a suborder of the rodents, are now placed by most mammalogists in a separate order, the Lagomorpha, which was proposed by Gidley (1912). The rabbits differ markedly from the true rodents in their parasites, diseases, and reactions to disease.

Tularemia is stated by most writers to be a rodent disease but practically all have failed to distinguish between true rodents and rabbits.

> Simpson (1929) states, "Tularemia exists primarily as an infectious disease of *wild rodents* particularly rabbits."
>
> Green (1931) says, "Tularemia is known to be a disease of rabbits as well as many *other rodents*."
>
> Hull (1941) writes of the disease, "primarily it occurs in nature as a fatal bacteremia of *wild rodents* especially hares and rabbits."
>
> Hagen (1943) repeats, "The disease affects *various rodents* especially the wild cottontail rabbit."

The anatomical distinctions usually cited which separate the rabbits from the rodents, the chief of which is the presence of an additional pair of incisor teeth immediately behind the large upper incisors, appear to be very minute. Hamilton (1943) states: "There is, however, enough difference between them and the rodents to justify regarding each as ordinally distinct, the similarity being accounted for through parallel development or convergence." In contrasting the rabbits and the rodents Ellerman in "The Family and Genera of Living Rodents" states, "The fundamental differences in the appearance of those parts of the skull to which the jaw muscles are attached may surely be at once stated in the Lagomorpha to be a much more important character than the retention of a functionless second upper incisor which seems to be quoted always as the main difference between the two groups."

Zoologists are unanimous in recognizing the difference between rabbits and rodents. Some, as Miller (1924), Scott (1937), Wilson (1937), Anthony (1928), and Hamilton (1939), place the rabbits in a separate order and others, as Kingsley (1912), Simpson (1931), Henderson and Craig (1932), and Hegner (1943), make only a subordinal distinction with the name Duplicidentata in contrast to the Simplicidentata or true rodents. Minor as these characters appear to be, they can be followed back through geological ages to the Oligocene (Gidley 1912, Dice 1929) or Paleocene (Simpson 1943), and so far no intermediate form has been found uniting the two groups. This antiquity is comparable to that of other orders of placental mammals according to a diagram prepared by Matthews (1943).

In a survey of the sources of human infection Francis (1937) says, "Wild rabbits and hares are the direct cause of over 90 per cent of the human cases in the United States."

In Illinois, which has reported twice as many cases of tularemia as any other state, "98.3 per cent of the cases were due to handling infected rabbits," according to McDaniels (1931).

Based on records of sources of human infection, tularemia in North America must be considered as primarily a disease of rabbits, of the order Lagomorpha, and secondarily as a disease of rodents, of the order Rodentia. Certainly less than 10 per cent and probably less than 5 per cent of the total cases are transmitted from rodents.

Obviously, all kinds of rabbits are not equally involved in the epidemiology of tularemia as emphasis has been placed on the cottontails, *Sylvilagus* spp., as reservoirs (Simpson 1929) while the domestic rabbit, *Oryctolagus cuniculus*, is conspicuously free of the disease (Francis 1925).

[1] Presented at the Fortieth Annual Meeting of the American Society of Tropical Medicine, St. Louis, Mo., November 13–16, 1944.

A brief synopsis of the rabbits of North America is taken from Miller (1924) whose classification will be followed in this paper. He lists two families, the Ochotonidae and Leporidae, as native to North America. These two families include all the living members of the order Lagomorpha. Both families have been monographed in the North American Fauna Series, "The Rabbits of North America" by E. W. Nelson (1909) and "Revision of the American Pikas" by Howell (1924). The family Ochotonidae contains but a single genus, *Ochotona*. Several subgenera are recognized in Asia but only the subgenus *Pika* is found in North America. The family Leporidae is represented in North America by four native genera, *Lepus*, *Sylvilagus*, *Brachylagus*, and *Romerolagus*. To the native North American rabbits must be added two introduced forms, the domestic rabbit, *Oryctolagus cuniculus*, and the European hare, *Lepus europaeus*. The latter has become established in nature in several areas in North America (Henderson and Craig 1932).

The American Lagomorpha are summarized as follows:

ORDER LAGOMORPHA

Family Ochotonidae

Genus *Ochotona*

25 species and subspecies.

Family Leporidae

Genus *Lepus*

120 native species and subspecies,
1 introduced species.

Genus *Sylvilagus*

45 species and subspecies.

Genus *Brachylagus*

1 species

Genus *Romerolagus*

1 species

Genus *Oryctolagus*

Introduced, many varieties.

Members of the family Ochotonidae are the smallest living rabbits, scarcely equaling a guinea pig in size. They inhabit talus slopes of the western mountains where they are known as rock rabbits, conies, little chief hares, and hay-makers. Their relationship to tularemia has not yet been determined as they have not been tested for susceptibility to *Pasteurella tularensis*, nor found infected in nature, nor suspected as a source of human cases.

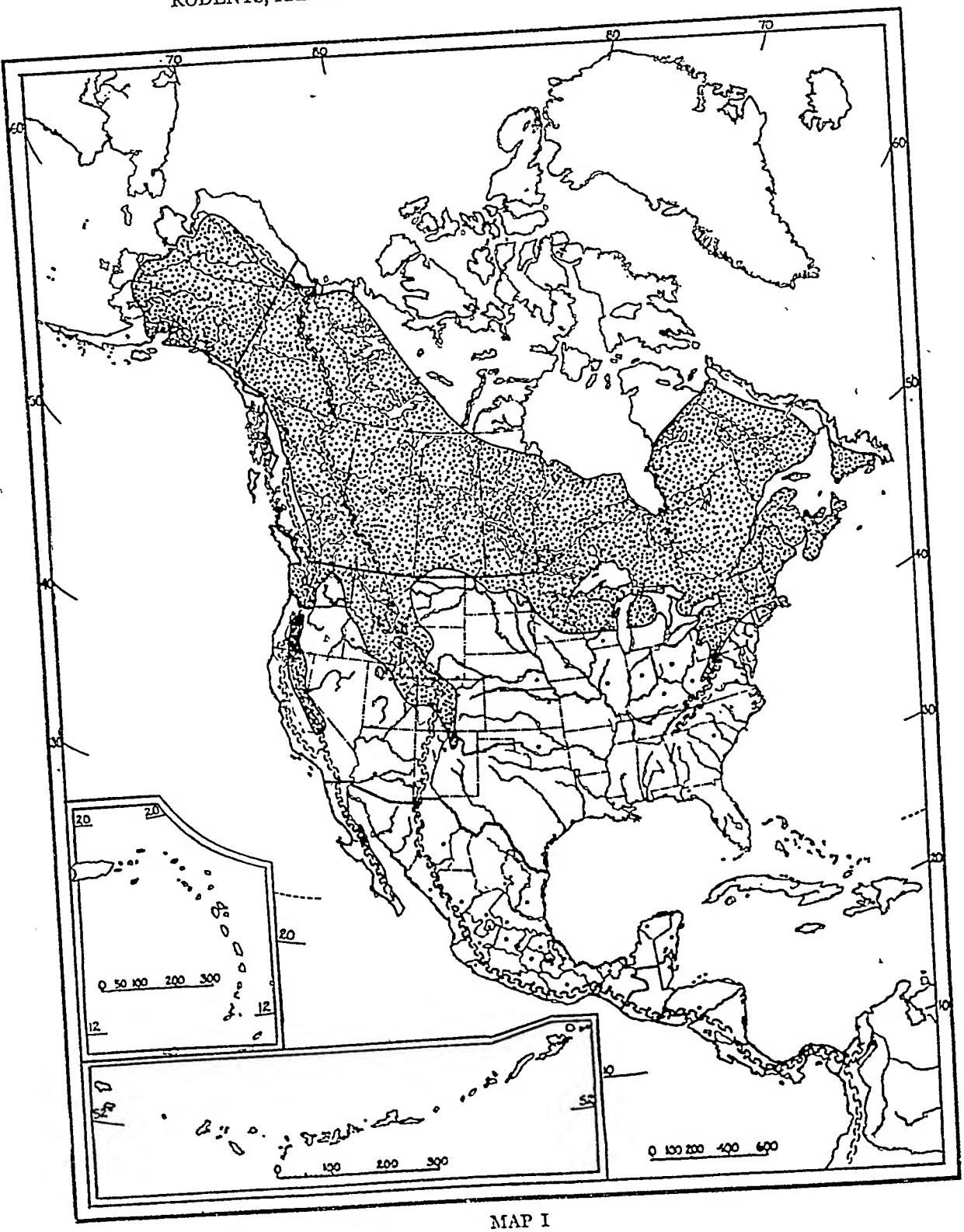
Several genera of the family Leporidae are of little significance as reservoirs of tularemia. The domestic rabbit, *Oryctolagus cuniculus*, a European species, though highly susceptible to the disease by laboratory experiment, has not been a proved source of human infection, except for laboratory workers. This anomaly is the subject of a paper by Francis (1925) entitled, "Absence of tularaemia (rabbit fever) among rabbits raised in rabbitries." On many farms the domestic rabbits are at liberty to range with their wild relatives and are more or less feral, still they seem to escape the disease.

A case of tularemia in northern Michigan thought to be due to contact with some type of domestic rabbit is reported by Belote (1931). The patient who had recently purchased the rabbits said they were represented to him as "chinchillas." Some disease was present in the rabbits as 7 of the 9 died within a few weeks time. A possible human case of tularemia from contact with domestic rabbits was reported to the Rocky Mountain Laboratory by Dr. E. P. Simms, Alamagordo, New Mexico. The patient, a laborer and resident of Alamagordo, became ill in October 1932 and Dr. Simms states the only source of infection as far as could be ascertained was from skinning a domestic rabbit. A recent letter from Dr. Simms (1944) states that diagnosis of this case was confirmed by an agglutination test.

The genus *Romerolagus*, or volcano-rabbit, is represented by a single species and is limited in distribution to a few mountain slopes in Mexico. It has not been implicated as a reservoir of tularemia.

The Idaho pigmy rabbit is given as a distinct genus by Miller (1924), but workers at the Museum of Vertebrate Zoology, University of California (Davis 1937 and others) consider it only a subgenus of *Sylvilagus*. Its range is centered in Idaho, but extends into the adjacent states of Nevada, California, Oregon and Montana. Although much smaller in size, it is seldom differentiated by local residents from cottontails which are present in the same area. It has not been tested for susceptibility to tularemia and it has not been specifically referred to in the literature as the source of human infection.

Two cases of tularemia possibly due to contact with a pigmy rabbit are reported in correspondence



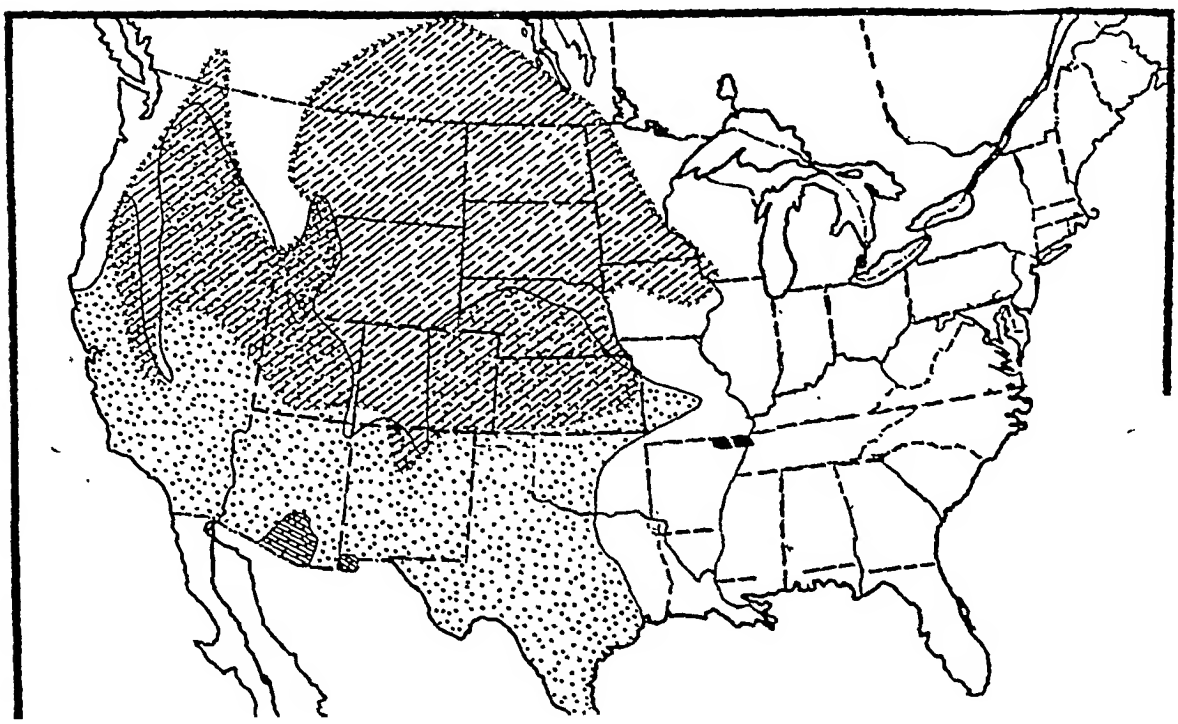
MAP I

RANGE OF VARYING HARES OF ALASKA, CANADA AND UNITED STATES
(After Seton, 1929 in part, and Kohls, 1940.)

(1944) by Mr. A. E. Borell of Albuquerque, New Mexico. While engaged in a study of the mammals of Nevada, which necessitated capture and preparation of many small animal skins, Mr. Borell and his brother captured a pigmy rabbit found in a lethargic condition near their camp in the Ruby Mountains of Nevada, July 22, 1927. After photographing the animal, it was prepared for the museum. (A picture of the rabbit has been published, Borell and Ellis, 1934. Within three weeks, Mr. Borell and his brother were ill of tularemia. Diagnosis of both cases was confirmed by agglutination tests. Three other local residents developed the disease about the same time. The work of preparing small mammal skins offered these men other chances of infection.

The genus *Lepus* is well represented in North America and includes: The arctic hares of Alaska and northern Canada; snowshoe or varying hares of northern Alaska, Canada, and the United States; and the jack rabbits of southern Canada, western United States and Mexico. (See distribution maps I and II.

The arctic hare, *Lepus arcticus* or related species, has not been found infected with tularemia or has its susceptibility been studied.



LEPUS TOWNSENDII GROUP. (AFTER NELSON, 1909 AND SETON, 1929.)

LEPUS CALIFORNICUS GROUP. (AFTER SETON, 1929, IN PART)

LEPUS ALLENI IN ARIZONA. (AFTER VORHIES AND TAYLOR, 1933) AND LEPUS GAILLARDI IN NEW MEXICO (AFTER BAILEY, 1931)

MAP II

RANGE OF JACK RABBITS OF UNITED STATES AND CANADA

(After Kohls, 1940.)

THE SNOWSHOE HARES

The varying hares or snowshoe rabbits,[2] *Lepus americanus*, *L. bairdii*, and *L. washingtonii*, inhabit almost the entire area of Alaska and Canada. They are extremely important members of the mammalian fauna, being used as human food and as a source of fur, as well as constituting the main food

[2] Dalquest (1942) considers all forms of the varying hare as subspecies of *Lepus americanus*.

supply for the more valuable fur bearing carnivores of the region. Their range in the United States is limited to the northern tier of states with more southerly extensions in the high mountainous areas.

The relationship of this group of rabbits to tularemia is deserving of detailed consideration.

Simpson (1929) states, "West of the Mississippi the jack rabbit (*Lepus*) and the snowshoe rabbit (*Lepus bairdii*), as well as the cottontail rabbit, are the most important direct or indirect agents in the transmission of the disease to man."

In a paper on "Sources of Infection and Seasonal Incidence of Tularemia in Man," Francis (1937) states, "Cottontail rabbit, *Sylvilagus floridanus;* jack rabbit, *Lepus* sp.; snowshoe hare, *Lepus bairdii;* these animals are the direct cause of over 90 percent of the human cases in the United States." However, he did not discuss their relative importance.

There are numerous papers on the cyclic abundance and scarcity of the snowshoe hare in Canada and the northern United States, and the effect of the rabbit cycle on the fur industry. As tularemia has been the most conspicuous disease known among wild rabbits, it has been generally assumed that tularemia was the cause of this periodic die-off. R. G. Green, of the Bacteriology Department of the University of Minnesota, and his associates have probably studied this cycle and its disease implications more continuously than any other workers. In 1935, Green and Shillinger stated, "The yearly census has shown a population decrease, and the mortality calculations given below present a picture of large losses among hares during 1933. It is only reasonable to ascribe to the observed spread of tularemia an important rôle in these losses, . . . recognizing the difficulty of finding carcasses in the young dense vegetation." And, "An unusual spread of tularemia among hares following a die-off of cottontails, seems to account for the decline." After 10 years of intensive field and laboratory study, they reached a different conclusion in 1939 that "Although tularemia is frequently seen in the hare, we have determined that it is not the cause of the die-off of the animal." Furthermore, they state, "We have found the snowshoe hare, which inhabits the northern half of Minnesota, to be resistant to tularemia. Rabbits of this species seldom die from the disease and most of them recover without becoming noticeably sick," but in regard to the cottontail, Green writes, "In the cottontail rabbit, which is found in the southern two-thirds of Minnesota, and which is very susceptible, it is a severe infection. Cottontail rabbits that have the disease do not generally live more than a week and invariably die of the infection."

Rather detailed monthly reports of the wildlife disease studies in Minnesota were prepared by Dr. Green and his associates and published as mimeographed circulars entitled "Minnesota Wildlife Disease Investigations." These deal largely with the snowshoe rabbit studies.

Conspicuous in these studies are the following:

1. The rarity of fatalities from tularemia among the snowshoe rabbits, even in fatal epizootics when many dead rabbits were examined. In July 1934, (1 animal); May 1935, (1 animal); June 1936, (3 animals); and June 1937, (1 animal); and possibly in other instances hares picked up sick or dead of tularemia were reported, and only in 1936 and 1937 were gross lesions characteristic of tularemia observed in these animals.

2. The absence of gross lesions in apparently normal rabbits, shot for examination, but in which tularemia was demonstrated by transfer of tissues to experimental animals.

3. The frequent presence of agglutinins for *Pasteurella tularensis* in trapped and apparently healthy hares.

In the Minnesota studies, Green frequently reported finding cottontails dead of tularemia and exhibiting gross lesions typical of the disease. In one instance (1932) a fatal epizootic occurred in cottontails in typical snowshoe territory but the only evidence of the disease in snowshoes was the presence of positive agglutinins in sera of these animals A contrast of the disease in the two hosts is given by Green (1938): "This difference between the pathological findings in cottontail rabbits and snowshoe hares seems to be a reflection of the severity of the disease in the two species. The cottontail rabbits, being highly susceptible to the disease, exhibited the characteristic lesions; while the snowshoe hares, more or less resistant to the infection, did not show a similar type of lesion under ordinary circumstances."

A study on the "Fluctuations in the numbers of the varying hares (*Lepus americanus*)" was made by MacLulich (1937) which included a historical, field and laboratory survey of the problem. He did not find tularemia infection in any of the varying hares but secured positive agglutination tests from 4 to 29 serum samples tested (with one titer as high as 1:5120). He states, "The decrease in abundance of hares was due to wholesale dying-off

. . . the epidemic is not always the same disease at every time and place."

Reference to distribution map I of the varying hare and map IV of the incidence of tularemia in man in North America shows there is very little relationship. The great bulk of the human cases occur south of the range of the varying hare. Reported cases of tularemia in Canada are not numerous and no definitely positive case has been reported for Alaska. The snowshoe rabbit is found in abundance in the northeastern states as Nelson (1909) quoted a statement that "about 2,000,000 varying hares are caught each winter in Maine," yet Francis (1937) states, "One is struck by the small number of cases occurring in the states comprising the northeastern section of the United States."

Comparison of distribution maps of the snowshoe rabbit and of tularemia incidence indicates, if anything, a lack of relationship between the presence of these rabbits and the presence of tularemia. Study of individual state maps emphasizes this lack of relationship. The snowshoe rabbit inhabits about three-fourths of the State of Washington, yet this is an area of low incidence. Its largest geographical range in the United States is in Idaho, western Montana, western Wyoming, northern Utah, and Colorado. Tularemia studies have been an important part of the research work of the Rocky Mountain Laboratory in western Montana, near the center of the area, since 1922, and extensive records on cases have been kept. We have only 3 human cases of tularemia recorded that were attributed to contact with the varying hares in these Western States. Two cases of tularemia due to contact with snowshoe hares at Carbarton Valley, Idaho, April 1929, were reported by Dr. J. C. Woodward of Payette. Both cases apparently handled the same rabbits; one case was confirmed by an agglutination test with a titer of 1:640. Another case probably from a snowshoe hare was reported by Dr. W. W. McKay from Utah, in March 1943. The prolonged incubation period, 30 days, after contact with "a white mountain hare" would suggest some doubt that this rabbit was the source of infection. (Recovery of infection from this species of rabbit in British Columbia, Canada, was reported by Parker, Hearle, and Bruce, 1931.) A single case in British Columbia due to the bite of a cat which had been fed a snowshoe rabbit found dead in the woods is reported by Moilliet (1936). The locality, 12 miles south of Kamloops, or about 130 miles north of the Canadian border, is well beyond the known range of rabbits other than snowshoes and conies.

THE JACK RABBITS

The jack rabbits, *Lepus* spp., occupy most of the area of the United States west of the Mississippi River. They extend into Canada in two separate places, a small area west of the Continental Divide in British Columbia and a much larger area east of the Divide in Alberta, Saskatchewan, and Manitoba. They range into southern Mexico but apparently not into South America. Map II shows their distribution. There are very few sections of much size where jack rabbits are present and cottontails entirely absent except in the extreme northern part of their range. Mature jack rabbits are sufficiently distinct in appearance to be recognized by the majority of people with any familiarity with animals so that case data specifying jack rabbits as the source of tularemia infection should be reliable.

In the original studies of "Deer Fly Fever" in Utah, Francis (1927) isolated tularemia from 17 jack rabbits, and later (1937) stated, "Cases west of the Mississippi due to the activities of skinning and cutting up wild jack rabbits for fish bait, coyote bait, chicken feed, dog feed, fox feed and for the table are without seasonal incidence."

Tularemia was reported from each of six lots of ticks from jack rabbits and from tissues of two white-tailed jack rabbits near Ringling, Montana, by Philip, Jellison and Wilkins (1935). One of the rabbits was found dead and lesions characteristic of tularemia were observed on the spleen and liver. Many other lots of ticks and tissues tested were negative. Infection in jack rabbits in southern Alberta has been reported by Brown (1943).

Two human cases, one fatal, due to contact with a sick jack rabbit in Minnesota were reported by Hartman, Beaver and Green (1933).

The only general State summary of tularemia cases in which the cases are designated specifically as to type of rabbit contact is by Brown, Lattimore and Hofman (1933) for Kansas, which states, "Of the 105 rabbit contact infections 98 were cottontails and 7 jack rabbits."

In most of the cases of tularemia reported to the Rocky Mountain Laboratory from the Western States in which rabbits were the source of infection, the type of rabbit responsible is not specified. However, among the numerous case records accumulated since 1923, in twenty-four jack rabbits are designated as the source of infection. Five of

these were confirmed by agglutination tests and the others are based on clinical diagnosis by the reporting physician. One case was fatal. These were distributed as follows: Idaho 5, Montana 2, Nevada 2, New Mexico 10, Oregon 3, Utah 1, and Wyoming 1.

The writers would not minimize the rôle played by jack rabbits, *Lepus* spp., as reservoirs of tularemia. Many human cases have come from direct contact with these animals and they are important hosts of ticks and probably of the biting flies that are known carriers of the disease. The jack rabbit is not hunted as intensively as the cottontail and in general occurs in areas of sparser human population.

THE COTTONTAIL AND RELATED FORMS

The genus *Sylvilagus* contains several groups of closely related rabbits including the tropical forest or swamp rabbits and the California brush rabbit, as well as the typical cottontails which include the eastern cottontail, *S. floridanus*, the New England cottontail, *S. transitionalis*, the western cottontail, *S. audubonii*, and the Rocky Mountain cottontail, *S. nuttallii*. Epidemiological data, other than geographical distribution, do not give separate consideration to the subgenera, species or subspecies.

The known northern boundary of the range of the cottontail in North America rather closely follows the Canadian-United States boundary. Some species are present in every state although their range in Maine is rather limited. They extend southward through Mexico, Central America, and South America to northern Patagonia, according to Nelson (1909). The genus *Sylvilagus* is not native in Asia or other continents of the Eastern Hemisphere.

Cottontails are extremely prolific and in spite of intense hunting and numerous natural enemies they maintain their numbers in well populated areas and even in the suburbs of cities. In the eastern United States, they are one of the most important game animals and are protected by closed seasons and bag limits.

The exact distribution of the cottontail is apparently extremely pertinent in the epidemiological study of tularemia in North America and appears to be especially critical in regard to hyman infections in the State of Washington and in southern Canada where cases appear to be just on the northern limit of the distribution of the genus. Seton (1929) in his section on the cottontails has a chapter entitled "Range and Northward March" and states, "But within 50 years the Eastern Cottontail has greatly extended its holdings northward—perforce northward, since it came from the south, and had already spread from sea to sea . . . it has steadily increased its territory as the snowshoe has retreated."

The extension of the range of the cottontail is also emphasized by Nelson (1909) who writes: "It is altogether probable that previous to the settlement of the country and its deforestation cottontails were unknown in a large part of the eastern United States."

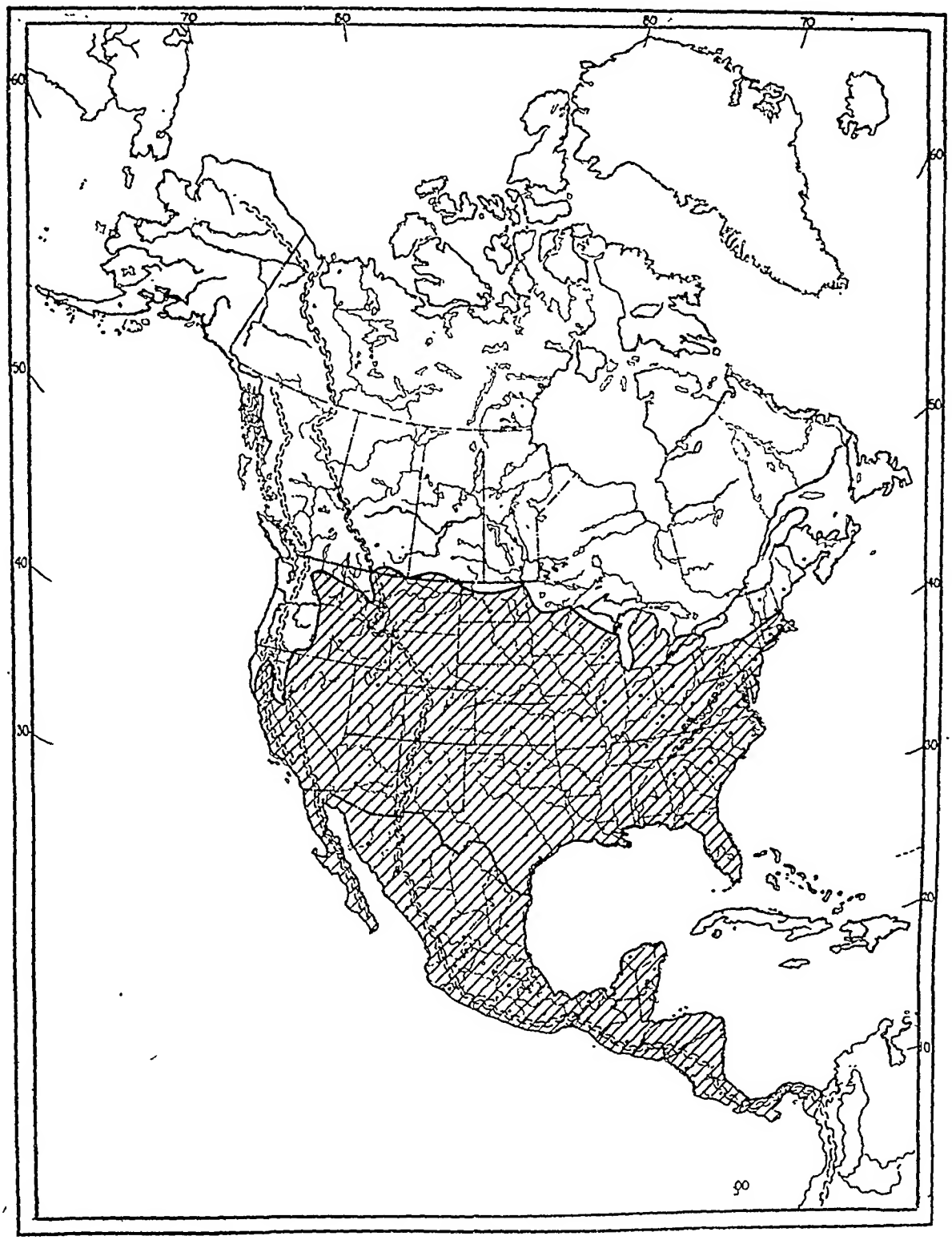
This genus of rabbits is directly responsible for the great majority of cases of tularemia within the United States. A distribution map of tularemia incidence was published by Alexander (1944) from data supplied by the Public Health Service and is reproduced here with slight modification and with the addition of Canadian cases. This invites comparison with the distribution maps of the various species of rabbits, and especially of the cottontail in the United States. Eleven states have reported 500 or more cases of tularemia per state. Ten of these lie south of the range of the varying hare, 9 lie east of the range of the jack rabbits, but all are within the range of *Sylvilagus* and specifically of *S. floridanus*. Except for other species of *Sylvilagis* no other rabbits are involved in this area. The 8 contiguous states where *Sylvilagus* is the only rabbit reservoir and tularemia case totals for each as given by Francis (1942) are as follows:

Illinois	2267	Indiana	483*
Ohio	1109	Louisiana	483*
Kentucky	936	Arkansas	409*
Georgia	610	Tennessee	409*

* Cases since 1942 apparently bring the total above 500.

Francis (1942) gives records by states of 14,002 cases of tularemia and not "13970" as indicated on his distribution map. Of these cases, 9,171 or 65.5 percent occurred in states practically free of all rabbits except cottontails. The remaining cases, 4,831 or 34.5 percent, occurred in states which do have cottontails but also have other genera of rabbits.

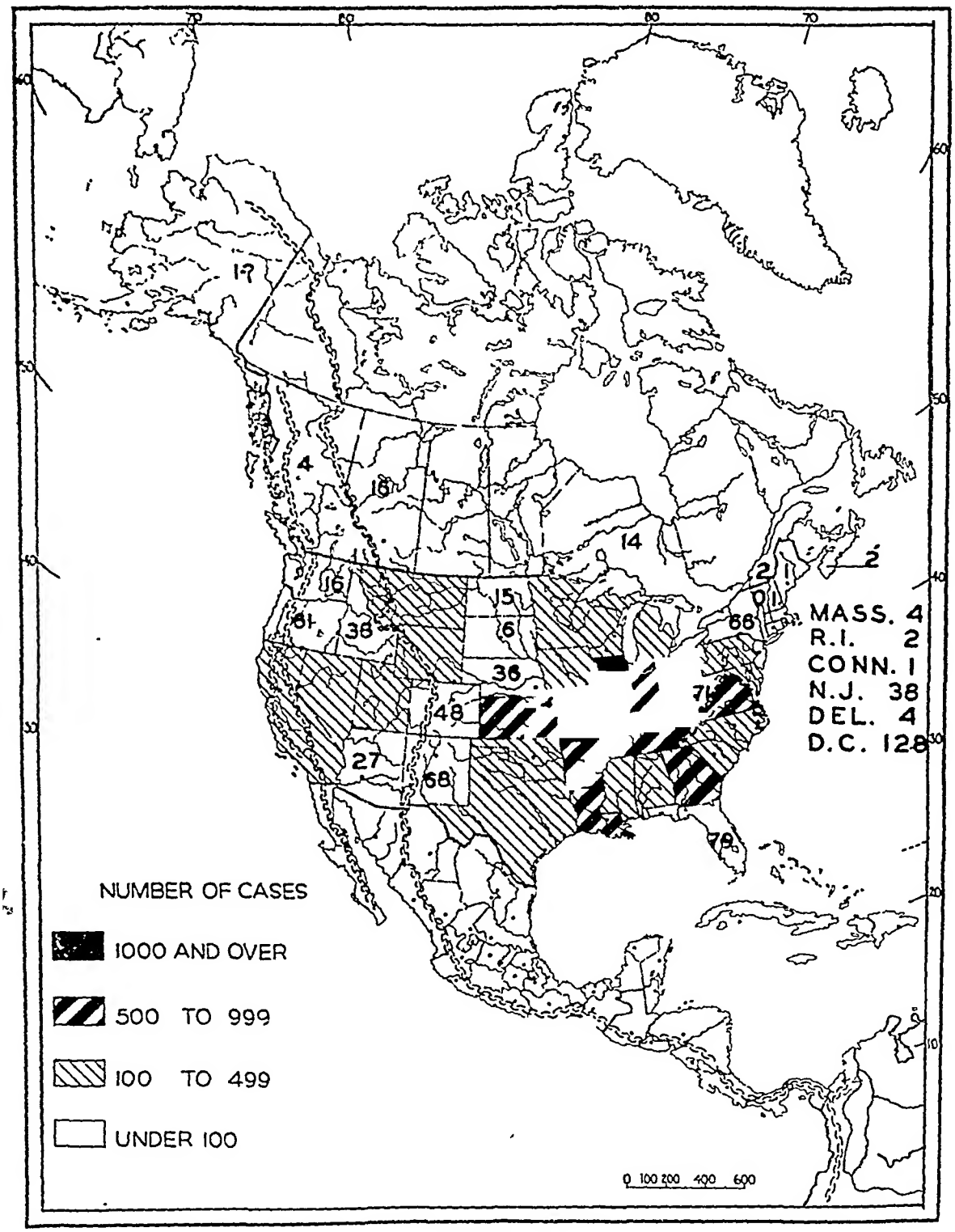
Illinois has reported more cases of tularemia than any other state—2267. The jack rabbit occurs only in the extreme northwestern corner of the State. *S. floridanus*, the only other wild rabbit in the State, is State-wide in distribution. McDan-



MAP III

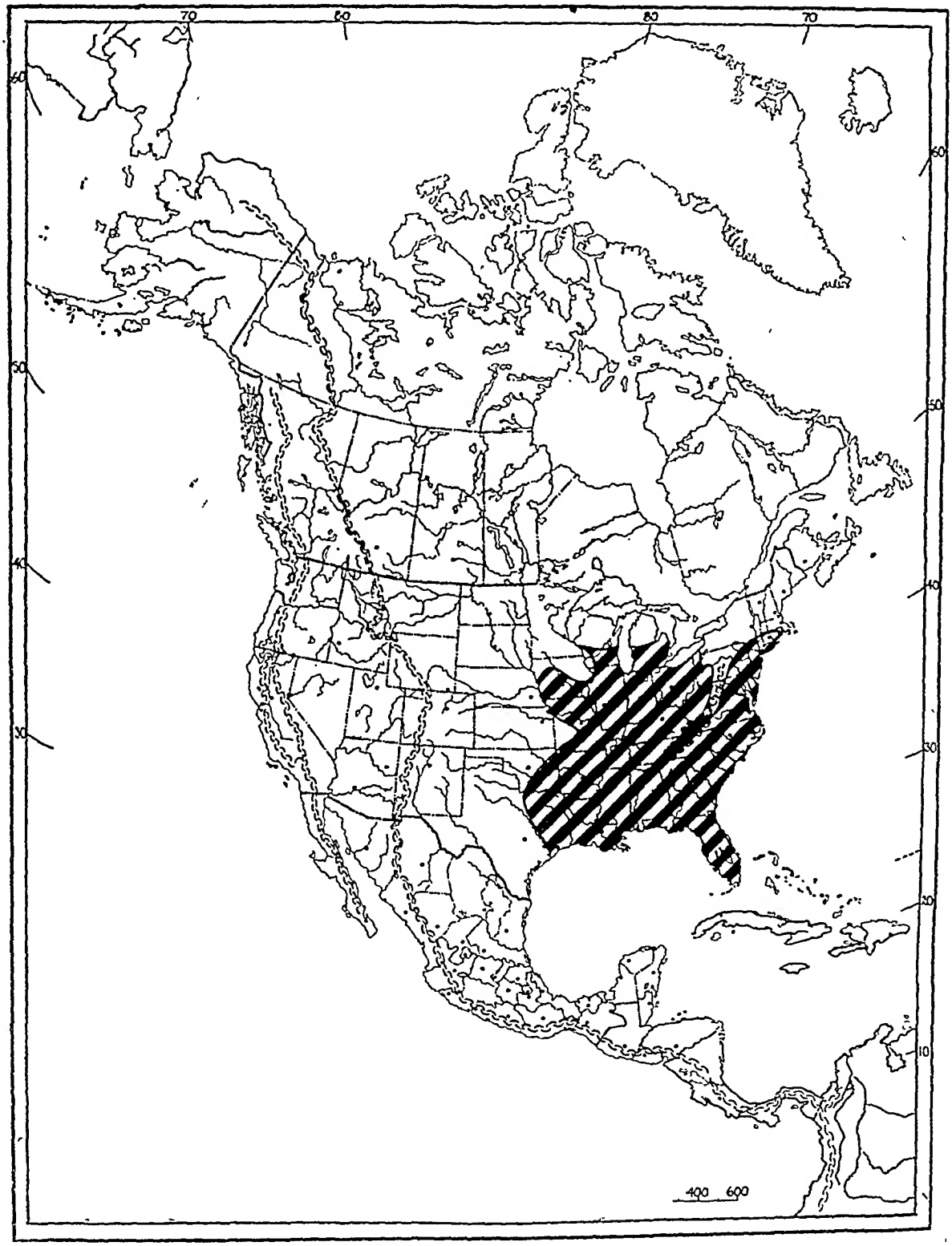
RANGE OF COTTONTAILS IN NORTH AMERICA

(After Seton, 1929, and Kohls, 1940.)



MAP IV

DISTRIBUTION OF TULAREMIA CASES IN NORTH AMERICA
(After Alexander, 1944, and Jenkins, 1939.)



MAP V

AREA OF UNITED STATES WHERE SYLVILAGUS IS THE ONLY GENUS OF WILD RABBIT PRESENT

iels (1931) estimated, "98.3 percent of the cases of tularemia in Illinois were due to handling infected rabbits," which could only have been native *S. floridanus* or rabbits shipped to the markets from other States.

Kansas, which borders on this area, does have an abundant jack rabbit population and a high incidence of tularemia—450 cases (Francis 1942). However, of 105 rabbit-contact infections from a total of 120 cases in Kansas reported by Brown, Lattimore and Hofman (1933), 98 were due to cottontails and only 7 to jack rabbits.

The scarcity of tularemia in the New England States has been cause for speculation. Francis (1942) recorded the following cases for this area:

Maine	1
New Hampshire	1
Vermont	0
Massachusetts	4
Connecticut	1
Rhode Island	1

Reference to distribution maps by Hamilton (1943), probably the most accurate available, shows that *S. floridanus*, the common eastern cottontail, extends only into Connecticut and not into the other New England States; however, it is reported in Massachusetts by Trippensee (1944). This rabbit does extend well into New York which reported 66 cases, although Francis (1937, 1944) states that most of the cases in New York were due to market rabbits shipped in from other states. Another cottontail, *S. transitionalis*, occurs in nearly all of the New England area except Maine. It is present only in the southern corner of this State.

Among the Western States, Washington has a fairly low incidence of tularemia, 16 cases. This is less than any other Western State except North and South Dakota. Published distribution maps show that only the southeast quarter of the state is occupied by cottontails, *Sylvilagus*, but information from the Washington Game Department indicates this range extends farther north than shown on the map. This is also evident on the map published by Dalquest (1941). Case records at the Rocky Mountain Laboratory show that of the 13 cases on which we have data, all occurred in the eastern half of Washington (Stevens County 3, Pend Oreille County 3, Lincoln County 3, Spokane County 3, Benton County 1). These counties lie just north of the range of *S. nuttallii* as given by Seton (1929), but within the present range of the cottontail. The area is also occupied by jack rabbits. A list of the tularemia cases in the State of Washington by counties since 1929 has been supplied by Dr. W. R. Geidt, epidemiologist (1944). Every one of the 21 cases reported occurred in counties in the eastern part of the State where cottontails are native, or in the few counties in the western part of the State where eastern cottontails, *S. floridanus*, have been established by sportsmen (Dalquest, 1941). Cases were attributed to contact with rabbits, muskrats, game birds and to the bites of flies and ticks.

Two species of cottontails occur in Oregon, *S. nuttallii* in the east and *S. bachmani* in the west. Francis (1942) records 61 cases of tularemia from Oregon. The Rocky Mountain Laboratory has case data on 36 cases. Thirty-five of these are within the range of *S. nuttallii* as given by Bailey (1936) in the "Mammals and Life Zones of Oregon," while only one case occurred within the range of *S. bachmani*, which occupies the coastal area. At least 8 recent cases in Oregon have been due to contact with muskrats, *Ondatra zibethica*, but in counties where cottontails were present.

The cottontail, *S. floridanus*, appears to be the only wild rabbit present in Ohio, a State that has reported the second highest tularemia incidence, 1109 cases according to Francis (1942), or 1,275 cases according to Hicks (1942). Hicks states that in Ohio, "More than 95 percent of the human cases come from contact with rabbits . . ." The seasonal incidence of the disease in Ohio coincides with the open season on cottontails, the most important game mammal in the State.

The distribution of tularemia in Canada must also be reconsidered in relation to *Sylvilagus*. The range of these rabbits in Canada is very limited. It comprises about 10 percent of Alberta, Saskatchewan, and Manitoba, and a separate area in Ontario and Quebec (Anderson, 1940). It also extends into a small area in southern British Columbia west of the Rocky Mountains according to Cowan and Hatter (1940). The cottontail is present in every State bordering Canada and its northern limit of distribution approximates the Canadian border. Data on tularemia in Canada were summarized by Jenkins (1939). The first case was reported in 1930. Up to 1939, Jenkins had records of 38 additional cases but stated, "My own belief is that the disease is much more common than our records would lead us to believe." These

39 cases were distributed as follows: Nova Scotia 2, Quebec 2, Ontario 15, Alberta 16, and British Columbia 4. This is a sharp contrast to a total of 2475 cases reported by Francis (1942) for the northern tier of States, Washington to Maine, or an average of over 190 cases per State. This contrast can hardly be attributed to population differences or to lack of diagnosis in Canada, but must be related to scarcity of the disease in nature and specifically to the absence of some reservoir hosts or hosts from which it is ordinarily contracted by man. Of the 39 cases reported by Jenkins, 37 were from provinces where the cottontail rabbit is known to be present. Nova Scotia with 2 cases is the only province or territory in North America reporting infection in man but not known to have cottontails.

An epidemic in cottontails at Kingston, Ontario, was studied by McLulich (1937). He did not establish the presence of tularemia but states, "It was practically proven that the epidemic was not due to tularemia."

Five cases of tularemia were reported from Ontario in 1932 by Johns (1933). All of these came from the vicinity of London in southern Ontario which is within the limited range of the cottontail rabbit in that province. However, none of the five were definitely due to direct contact with rabbits. Three appeared to have been contracted from muskrats.

Of tularemia in Alberta, Brown (1943) states: "Since 1938 the Alberta Rocky Mountain spotted fever survey has shown tularemia infection to be widespread in southern Alberta, south of township 24, being particularly bad in the extreme southeastern part of the province." It is emphasized that the southeastern part of the province is the only part occupied by *Sylvilagus* according to distribution maps available.

It would be very desirable to restudy the Canadian cases of tularemia with respect to specific source of infection, and geographical locality and also to know the exact distribution of *Sylvilagus* in Canada.

Alaska is entirely beyond the range of the cottontail rabbit and so far no definite human case has been reported for that territory. Philip (1939) reported a suspected case and recorded four isolations of *P. tularensis* from rabbit ticks from snowshoe hares in Alaska.

Although the range of *Sylvilagus* extends southward through Mexico and South America, no record of tularemia in Mexico or South America was given by Francis (1942). Mexico is listed by Jackson (1942) as one of the countries from which tularemia has been reported but he cites Top (1941) as the authority for the statement. It is evidently an error as reference to this publication does not verify the statement. More recently (1944) tularemia has been reported in Mexico by Tovar. California, Arizona, New Mexico, and Texas which border Mexico have reported 246, 27, 68, and 384 cases of tularemia, respectively, but a distribution map of cases by Schultze and Marr (1934) for Texas shows very few cases near the Mexican border.

A comparative study of the virulence of strains of tularemia isolated from snowshoes, cottontails, and grouse was made by Green (1943). The standard of measure of virulence was half-day survival of guinea pigs inoculated with freshly isolated strains. Data were analyzed mathematically and X^2 [chi] calculated. This analysis indicated that the observed differences were significant. The strains studied were virulent in the following order: from *Sylvilagus* most virulent, inoculated animals surviving 10 half days; from *Lepus americanus*, moderate virulence, animals surviving 18 half days; and from grouse, *Bonassa*, least virulent, animals surviving 30 half days. The virulence for strains isolated from *Lepus americanus* is similar to strains isolated from jack rabbits, *Lepus townsendii*, by Philip, Jellison and Wilkens (1935).

No tularemia data are available to separate the swamp rabbits and marsh rabbits, subgenus *Tapeti* of the genus *Sylvilagus*, from the true cottontails. All states occupied by the *Tapeti* group are also within the range of the cottontails. State reports do not distinguish them as a separate source of tularemia. Southern States within the ranges of both rabbits have reported a considerable amount of tularemia—Georgia, 610 cases; Alabama, 165 cases; Mississippi, 100 cases; Louisiana, 483 cases. The subgenus *Tapeti* has a greater range in South America than the subgenus *Sylvilagus*, which occupies only a limited zrea. Tularemia has not been reported for South America and this may be due to scarcity of true cottontails. Little is known of the diseases or parasites of rabbits of the subgenus *Tapeti*.

CONCLUSIONS

1. In the epidemiology of tularemia the distinction between rabbits, the order Lagomorpha, and rodents, the order Rodentia, should be recognized.

2. About 90 per cent of the human cases of tularemia result from contact with rabbits.

3. As the remaining 10 per cent must include cases contracted from rodents, sheep, game birds, miscellaneous mammals, as well as tick and deerfly bites, it is obvious that less than 10 per cent of the cases are the result of contact with true rodents.

4. The importance of the rock rabbit, *Ochotona*, the volcano rabbit, *Romerolagus*, the pigmy rabbit, *Brachylagus*, the arctic hare, *Lepus arcticus*, and the domestic rabbit, *Oryctolagus*, as reservoirs or sources of human infection is negligible or unknown.

5. The snowshoe, *Lepus americanus*, has been found infected in nature, but its importance as a source of human infection has been overemphasized.

6. The jack rabbits are known reservoirs and the source of a small percentage of human cases of tularemia. Their greatest importance may be indirect as a source of infection for ticks and deerflies which later bite and infect man.

7. In North America the cottontail rabbits, *Sylvilagus* spp., and in particular *S. floridanus*, are the direct source of over 70 per cent of all human cases of tularemia. Of the 14,000 total cases reported, less than 40, or 0.3 per cent, occurred beyond the known range of *Sylvilagus*.

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A CLINICAL CURE OF MADURA FOOT

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The experience with some newer medicaments in the treatment of Madura Foot may be of importance for the patients suffering from this disease. The advice, given in all latest editions of textbooks about the therapy of Madura foot, namely amputation, was a stimulation for the author to investigate first the influence of three newer drugs before pronouncing that sentence.

The patient W. T. a sturdy colored man, 53 years of age, farm-laborer in a village in Louisiana, was admitted to the Charity Hospital, New Orleans, on January 3, 1944. His chief complaint was swelling of the left foot with occasionally moderate pain. In 1942 he noted a swelling in the sole of the left foot. This swelling grew larger and was soon followed by the appearance of openings covered with crusts. The patient had never been sick before. He often stood barefooted in water, digging ditches for irrigating purposes. The family history was irrelevant. Upon physical examination no pathological findings were observed, except in the left foot. The swollen area was covered with numerous crusts closing the sinustracts and surrounded by a very dark pigmentation (fig. 1). On removing a crust, a purulent fluid containing small yellowish granules came out. In this case, thanks to the examinations by Dr. Edward Burns, pathologist, and his assistant Mr. J. Brueck, an Actinomyces was cultured from the yellow granules found in the pus of the sinustracts. The organism appeared to be *Actinomyces asteroides* (Eppinger-Gasperini), an anaerobic organism dis covered in 1894 and found in a case of Madura Foot by Castellani (1). Urine and blood of the patient showed no pathological findings. Kline and Kolmer tests were negative. The Roentgenphoto of the left foot shows decalcification of the bones with small punched out areas at the bases of the 3rd and 4th metatarsal bones and at the distal end of the 4th metatarsal bone. In reports of nearly all the cases of Madura Foot, the author has found a history of walking barefoot, also in patients of higher social standing sometimes during fishing trips.

A few words about the etiologic agents may be added because the commonest cause of Madura Foot in the United States is not an *Actinomyces*. As early as 1913, Pinoy, of the Institut Pasteur in Paris, discovered that this disease may be caused by two groups of organisms, an *Actinomyces* group and a true fungus group. Today we know that the etiologic agent may be one of 13 species of *Actinomyces* or one of 19 species of true fungi. The true fungi belong to two classes and eight genera. The striking similarity of the clinical picture indicates that there must be a common denominator, e.g. the production of a toxin of the same chemical structure or at least toxins producing the same pathological effects in the tissues. The synonym Mycetoma, given by Carter in 1860 for Madura Foot whose clinical picture was already described in 1842 by Gill in Madura (India), should not be forgotten because this disease may develop rarely on the hands or knees. The usual cause of Madura Foot in the United States is a true fungus, the *Monosporium apiospermum*. In 1931 only two cases of *Actinomyces* Madura Foot were collected by Jones and Alden (2). Of the seven cases of Madura Foot in the United States which have been published since 1931, only one was considered by Dixon (3), to have been caused by an *Actinomyces*, but this was not proven by cultures which are absolutely necessary in order to make that diagnosis. Moreover, claims of success with a therapy in this disease requires cultures. Gellman et al. (4), Brindley et al. (5) and Feinberg (6) described cases where *Monosporium apiospermum* was cultured. Feinberg's patient had never travelled beyond the limits of the United States and Canada. The patient, herein reported, living in Louisiana, had never traveled farther than Texas. It is probably the third case of *Actinomyces*-Madura Foot, proven by cultures, in the United States.

The therapy consisted in treatments first with penicillin, later with sodium propionate and still later with sulfadiazine.

Penicillin was given in large doses over a period of four weeks. The patient received during this

period 1,050,000 Units. The *Actinomyces* grew just as rapidly as if saline had been injected.

Because sodium propionate, according to Keeny (7), inhibits the growth of several pathogenic true fungi and some species of Actinomyces (e.g. *Actinomyces bovis*), it was tried in this case of Madura Foot. Dressings of 20 per cent sodium propionate were prescribed and isotonic solutions of the drug were injected in the sinus-tracts. The *Actinomyces* grew just as well with or without sodium propionate.

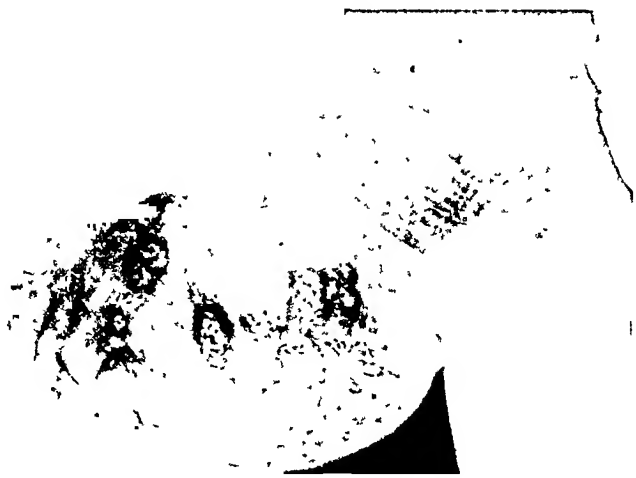

FIG. 1. BEFORE TREATMENT

FIG. 2. AFTER TREATMENT

Iodides were not given by the writer, although he remembers the enthusiastic reception of the iodide therapy for actinomycosis, as the veterinarian Dekhuizen in Utrecht demonstrated the more rapid recoveries of cervico-facial actinomycosis with this drug in man and cattle. The Madura Foot-species of *Actinomyces* are so resistant to iodides that this therapy has always been followed by amputation.

Sulfonamides were tried by the writer as an ultimum refugium, before delivering the patient to the surgeon. The intention was to give it in large amounts and for a long period, under severe control. The disease of Nicolas-Favre had taught us the importance of sulfonamide-dosages. The writer could not find in the literature the description of a case of *Actinomyces*-Madura Foot, *proven by cultures*, which was clinically cured by sulfonamides. This was confirmed by several specialists in the field of subtropical and tropical diseases (McCoy, Napier, Faust). Temporary improvements, but no clinical cure, were observed from sulfonamides in other parts of the body which were infected with *Actinomyces* (Poulton, Walker, McCharles, Kippen, Miller and Fell). Cases of lung-actinomycosis which showed such temporary improvements, have been described recently by Benbow et al. (10). Lung-actinomycosis is caused in 90 per cent by *Actinomyces bovis* and in nearly 10 per cent by an aerobic *Actinomyces*-species, called *Nocardia*. Different species of *Actinomyces* probably show different resistance against sulfonamides. However the writer would advise treating all species with higher doses of the drug and over longer periods.

This case of Madura Foot was given sulfadiazine in doses which kept the blood-level during the daytime at 8 mgm. per cent, e.g. 1 gram every 4 hours during the daytime with a double dose of sodium bicarbonate and two liters of water every day. The drug was given over a period of three weeks

and the patient was kept under careful observation. The urine was examined every day and the blood twice a week. The drug was tolerated very well. The blood-levels during the daytime were always about 8 mgm. per cent. The slight fever, which the patient had continuously during the treatments with penicillin and sodium propionate, disappeared completely after one week of sulfadiazine-administration. This sign of becoming fever-free after one week of sulfonamide-administration may be of importance for the prognosis. The first culture after the three-weeks' treatment was negative. It was taken from the open sinus-tracts whose number was more than 25. A second culture, a few days later, revealed a slow growth. There was apparently present a fungistatic action of the sulfadiazine. Therefore, the writer wanted to begin with a second course of the sulfa drug, but the patient felt so much better after disappearance of the fever and pain, the moderate decrease of the swelling of the foot that he wished to go home. The author prescribed, for use at home, small doses of the less toxic methyl-sulfadiazine (sulfamerizine) with all of the already mentioned precautions, as far as possible, but it was not taken, pecuniae causa. For the same reason he did not return from his small town in the North of Louisiana until the writer asked him urgently in a letter to return, in order to check the results. This was about half a year after his discharge.

At this time it was striking to see a foot of normal size as compared with the other foot. No sinus-tracts were left. A culture from its contents was for that reason impossible. The patient not only walked, which was impossible for more than one year but again did all his work. Several clinicians who had seen the foot before, agreed that this might be called a "clinical cure." (Compare fig. 1 with fig. 2). The term "clinical cure" has to be understood as it is in a case of a so-called clinical cure of lung-tuberculosis. This accepted terminology does not exclude that sometimes in old calcified tuberculous lesions of the lungs living tubercle bacilli may be found by animal inoculation, if the patient dies from other causes than tuberculosis. Only the future can answer the question if a similar phenomenon may be expected in clinically cured cases of Madura Foot.

The statements of Strong (2) and Manson-Bahr (3) in the latest editions of their textbooks of tropical diseases, namely that the only effective treatment of Madura Foot is amputation, apparently no longer hold true. Probably the fungistatic action of the sulfonamides is a sufficient support to aid Nature in her attempt to cure this infection. This is analogous to the results of the well-known bacteriostatic action of these drugs.

SUMMARY

A third or perhaps fourth case of Madura Foot, caused by a species of *Actinomyces*, is described in a man who never left the United States. It is about the fortieth case of Madura Foot if cases caused by true fungi are included. A great number are probably never recognized but are treated as chronic osteomyelitis, sarcoma tuberculosis or syphilis. The writer knows three such cases, where the diagnosis was made following amputation. Treatment with penicillin and sodium propionate had no effect whatsoever, but sulfadiazine showed a fungistatic action, beginning with the disappearance of the fever and developing into a clinical cure.

The author's conclusion is that, from now on, every case of *Actinomyces* Madura Foot should be treated with doses of sulfonamides, sufficient to produce blood-levels of about 8 mgm. per cent, during at least three weeks and, if necessary, with repeated courses of this therapy. Advice to amputate the member should never be given before this is seriously tried. Because of the great similarity of the clinical picture of the cases of Madura Foot caused by *Actinomyces* and those caused by true fungi, the benefit of a trial with all the precautions mentioned above, should be given to every patient suffering from Madura Foot.

P.S. In July 1945 the old-patient came, on my request, in my office for controle. It was more than one year after his discharge. The foot showed a complete clinical cure. The man does all his heavy work as before the disease.

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THE ACID-ETHER CENTRIFUGATION AND THE ZINC SULFATE FLOTATION TECHNIQUES AS METHODS FOR THE RECOVERY OF THE EGGS OF SCHISTOSOMA MANSONI[1]

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The diagnosis of *Schistosoma mansoni* infection is, of necessity, based on the demonstration of the characteristic lateral spined eggs in the feces. The demonstration of these eggs involves problems not encountered in the isolation of the eggs of the majority of the intraluminal intestinal helminth parasites of man. At certain stages in intestinal schistosomiasis relatively few eggs are passed and concentration techniques are therefore necessary for their isolation. As schistosome eggs do not float in concentrated saline solutions, the commonly used brine concentration methods are not applicable. In formed stools, schistosome eggs are most numerous in the mucous layer on the surface of the stool, and may be absent in the center of the fecal mass (Khalil and Salah el Din, 1930). Recognition of these differences has led to the development of specialized isolation techniques. Certain of these, such as the hatching technique of Fülleborn (1921) and the rectal swab method of Khalil and Salah el Din have proven to be efficient diagnostic procedures for the investigation of small groups of patients, but are not readily adaptable to routine use in large scale studies. Scott (1937) reviewed the various methods utilized in field surveys for the isolation of the eggs of *S. mansoni* and stated that "there is still a need for some new procedure applicable to field conditions which will give high degree of accuracy in the estimation of incidence at low cost."

The present paper is a report of studies carried out in an attempt to evaluate and compare the effectiveness of the acid-ether centrifugation and the zinc sulfate flotation techniques in the isolation of schistosome eggs. The primary purpose of the study was to evaluate an acid-ether centrifugation technique which was adopted by our laboratory at the suggestion of the late Dr. W. A. Hoffman of the School of Tropical Medicine, San Juan, Puerto Rico. It was applied during a survey of a large group of Selective Service registrants and appeared to be a rapid and simple means of concentrating schistosome eggs.

The acid-ether centrifugation method was introduced by Telemann in 1908, who emulsified fecal material in equal parts of ether and hydrochloric acid. Shortly thereafter, Pfister (1909) found the Telemann technique to be useful for the concentration of schistosome eggs and included it as a routine procedure in the examination of suspected cases of schistosomiasis. Fülleborn (1921) also recommended the acid-ether method, but used a 50% concentration of hydrochloric acid. More recently the original acid-ether method has been modified by the substitution of other acids, such as glacial acetic acid (De Rivas, 1928). Faust et al. (1938) (1939) introduced zinc sulfate solution of a specific gravity of 1.180 as an agent that would float helminth eggs and protozoan cysts without causing distortion. Although it has been stated that this technique is satisfactory for the recovery of schistosome eggs, no detailed report of its effectiveness can be found in literature.

MATERIALS AND METHODS

The material studied was obtained from Puerto Rican Selective Service registrants who, in the course of routine fecal examination, had been found to have schistosomiasis. Because the egg output was low in most of these individuals, a stool specimen was collected from the occasional registrant found to be passing relatively large numbers of eggs. The specimens were collected in one-half pint Mason jars and placed in a refrigerator (5°C.) within two to four hours after collection. The majority were examined within 24 hours of receipt, but an occasional specimen remained in the refrigerator for 5 days before being studied; a record was kept of the time elapsing before examination. Each specimen studied was obtained from a different individual.

In brief, the procedure consisted of preparing a uniform suspension (termed the basic suspension)

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of each specimen of feces. The number of eggs per cc. in the basic suspension was established by the Stoll egg-counting technique, and then the number of eggs recovered from the suspension by each of four methods of concentration was determined. The following procedure was employed in the examination of all coverslip preparations. The schistosome eggs in each preparation were counted by two observers who systematically surveyed the whole area of the coverslip with the aid of a mechanical stage, using a magnification of 100×. Records were kept of the appearance of the ova. All coverslips were ringed with vaseline before use to prevent escape of eggs.

1) *Preparation of basic suspension:* The stool specimen was stirred with a spatula. A 20 gm. portion was transferred to an evaporating dish and 20 cc. of 0.85% NaCl solution were added. The mixture was thoroughly emulsified by stirring until it appeared homogeneous. It was further stirred before each sample was withdrawn. The desired amount to be tested was transferred using a 10 cc. pipette. Although the basic suspension approximated a 50% concentration of the original fecal specimen by weight it was arbitrarily considered to represent a 50% suspension by volume and all egg counts were expressed in terms of eggs per cc. of the original specimen.

2) *Determination of number of schistosome eggs per cubic centimeter of basic suspension:* The displacement dilution egg counting technique of Stoll and Hausheer (1926) was employed with the following modification. Twice the usual volume of fecal material was employed, so that 52 cc. of N/10 NaOH were pipetted into the egg count flask and basic suspension was then added to the precalibrated 60 cc. mark. After shaking, three 0.075 cc. samples were withdrawn with a Stoll pipette; each sample was placed on a 22 x 40 mm. coverslip, a 2 x 3 inch slide superimposed and examined by two observers. The six counts were averaged, and the result multiplied by 200 to express the result in terms of eggs per cc. of feces.

3) *Acid-ether "routine" technique:* This method differed from the survey technique used in the laboratory only in the manner of selection of the fecal sample to be tested. In survey work, a pea-sized fragment of feces was emulsified in 5 cc. of 40% HCl (40 cc. conc. HCl diluted to 100 cc.), while in the present study one cc. of the basic suspension was pipetted into a 15 cc. centrifuge tube, 5 cc. of 40% HCl were added, and the material was mixed by shaking. The material was filtered through two layers of moist gauze stretched over the top of a 50 mm. funnel into a second tube. (In this step, and in the techniques described below, the filter was composed of two three inch squares of Bay's (Parke Davis and Co.) type VII, 20-12 grade gauze.) An equal quantity of ether was added, the tube was stoppered with a gloved thumb and shaken thoroughly. It was then spun in a horizontal centrifuge for one minute at 1500 R.P.M. Upon removal from the centrifuge, the debris floating at the acid-ether junction was loosened by ringing with a clean applicator stick, and the acid and ether layers were rapidly poured off and discarded. The same applicator stick was used to stir up the sediment in the few drops of fluid remaining. The sediment was decanted onto a slide, and a ringed 22 x 22 mm. coverslip applied. The counts of the two observers were averaged and the result multiplied by two.

4) *Zinc sulfate flotation with loop removal:* The method used was essentially as outlined by Faust et al. (1939). One cc. of the basic suspension was pipetted into a Wassermann tube; 6 cc. of tap water were added and the contents thoroughly mixed. The material was filtered (as described above) into a second Wassermann tube. Tap water was added until the fluid level was within one centimeter of the top of the tube, the contents were mixed and the tube spun in a horizontal centrifuge for 45 seconds at 2640 R.P.M., after which the supernatant was poured off and discarded. Tap water was again added and the process repeated; the washing procedure was always carried out three times, and occasionally as many as five times. One cc. of zinc sulfate solution of a specific gravity of 1.180 was added and mixed with the sediment by shaking. Additional $ZnSO_4$ solution was added to within 0.3 cm. of the top of the tube and the tube recentrifuged for 45 seconds at 2640 R.P.M. Three, and occasionally four, loopfuls of the material floating on the surface were then transferred to a 22 mm. square coverslip using a 5 mm. bacteriological loop. Each loopful was picked up by inserting the loop vertically near the side of the tube and then picking up a portion of the film as described by Faust and his co-workers. A slide was superimposed on the coverslip. The two counts were averaged and multiplied by two.

5) *Acid-ether semiquantitative technque:* One cc. of basic suspension was pipetted into a centrifuge tube and 5 cc. of 40% HCl added; the emulsified

material was then handled as described under the acid-ether "routine" technique through the centrigugation step. Following centrifugation, the ether layer, the debris floating on the acid layer, and part of the acid layer were successively withdrawn by the introduction of a pipette attached to a water suction pump. The acid layer was removed until the meniscus was at a previously calibrated one cc. mark. The sediment was then mixed with the fluid remaining, and three 0.075 cc. samples were withdrawn with a Stoll pipette. Each sample was placed on a ringed 22 x 40 mm. coverslip, a 2 x 3 inch slide superimposed and examined by two observers. The results of the six counts were averaged, and this figure was multiplied by 26.7 to express the answer in terms of ova per cc. of undiluted feces.

6) *Zinc sulfate semiquantitative technique:* The method used was similar to that described by Palmer (1941) and Sawitz (1942). One cc. of basic suspension was pipetted into a Wassermann tube, the rim of which had been ground flat. It was filtered and washed as described in the section on zinc sulfate flotation with loop removal. The final addition of $ZnSO_4$ solution (sp. gr. 1.180) was made so that the meniscus was level with the top of the tube. A 22 x 22 mm. coverslip was placed on the top of the tube and the tube was centrifuged for 45 seconds at 2640 R.P.M. The coverslip was removed with a sharp upward lift, inverted, and a slide superimposed. The fluid in the tube was again brought to the top by the addition of 2 to 3 drops of $ZnSO_4$ solution, a coverslip applied, and the process repeated. A third preparation was made in the same manner. The two counts on each preparation were averaged, the average counts for the three coverslips were added, and the result multiplied by two. The number of eggs remaining in the sediment was next determined. The zinc sulfate solution was withdrawn to a previously calibrated two cc. mark by the gradual introduction of a capillary pipette attached to a water suction pump. The sediment was thoroughly mixed with the fluid remaining. A 0.075 cc. drop was then placed on each of two 22 x 40 mm. ringed coverslips, using a Stoll pipette. The four counts were averaged, and the result multiplied by 53.3.

RESULTS

Forty-six specimens were examined by each of the techniques outlined above. The results are summarized in table 1. Twenty-five of the specimens were found to contain more than 400 eggs per cc. by the Stoll count; calculations of the percentage recovery of available eggs were limited to this group.

All 46 specimens were positive by the acid-ether routine technique. In the group of 25 specimens containing more than 400 eggs per cc., this method gave an average recovery of 20.6% of the available eggs with the percentage of recovery in individual specimens varying from 8% to 42% (see fig. 1). The zinc sulfate loop removal technique recovered schistosome eggs from 35 of the 46 specimens. In the group of 25 specimens loop removal gave an average recovery of 4.6% of the available eggs with variation in individual specimens from 0% to 24%. On the basis of total number of eggs recovered, the acid-ether diagnostic method was superior in 41 specimens, the zinc sulfate technique in 4 specimens, and the two methods recovered an equal number of eggs from one specimen.

The acid-ether semiquantitative technique recovered schistosome eggs from 44 of the 46 specimens. In the group of 25 specimens, the percentage recovery of available eggs varied from 9% to 105%, with an average percentage of recovery of 48.2%. The zinc sulfate semiquantitative technique recovered eggs from 43 of the 46 specimens. In the group of 25 specimens, the recovery of available eggs varied from 0.2% to 72%, with an average percentage of recovery of 22.2%. Eggs were recovered from the sediment remaining in the zinc sulfate tube in 41 of 45 specimens. In 33 of these, the calculated number of eggs present in the sediment was greater than the number recovered on the superimposed coverslips. The percentage of available eggs remaining in the sediment was calculated for 24 specimens; the average for the group was 39% with a variation in individual specimens from 5% to 106%.

The eggs recovered by the acid-ether techniques were not distorted. In many, but not in all, of the zinc sulfate preparations, the schistosome eggs showed varying degrees of shrinkage and distortion, although they were still recognizable by the experienced technician. Empty egg shells were present in small numbers in both the acid-ether and zinc sulfate preparations. No correlation was noted between the appearance or the efficiency of recovery of the eggs and the length of time the specimen was refrigerated before being examined. The majority of the acid-ether preparations contained less extraneous fecal debris than the zinc sulfate

TABLE 1

*Summary of Counts of Schistosome Ova Recovered by the Acid-Ether and Zinc Suflate Techniques and of the Percentage of Recovery of Available Eggs**

SPECIMEN NO.	STOLL COUNT EGGS PER CC.	DIAGNOSIS TESTS				SEMI-QUANTITATIVE TESTS													
		Acid-Ether Routine		$ZnSO_4$ Loop Removal		Acid-ether					Zinc Sulphate Multiple Coverslip								
						Sample					Supernatant					Sediment			
											Cover Slip					Sample			
		Eggs per cc.	% recov.	Eggs per cc.	% recov.	I	II	III	Eggs per cc.	% recov.	I	II	III	Eggs per cc.	% reccov.	I	II	Eggs per cc	% recov.
1	2133	258	*12*	508	*24*	49	45	43	1231	*58*	362	37	2	803	*38*	13	25	1027	*48*
2	1833	154	*8*	133	*7*	18	23	21	555	*30*	212	12	0	448	*24*	1	3	107	*6*
3	1667	139	*8*	14	*0.8*	6	11	12	267	*16*	96	36	6	278	*17*	9	5	399	*24*
4	1567	190	*12*	41	*3*	17	21	25	574	*37*	66	23	0	180	*11*	17	11	773	*49*
5	1400	271	*19*	223	*14*	3	10	18	286	*20*	171	9	4	343	*24*	7	9	426	*30*
6	1367	244	*18*	8	*0.5*	13	5	19	379	*28*	44	2	1	96	*7*	12	9	576	*42*
7	1333	269	*21*	12	*0.9*	4	6	11	192	*14*	61	0	0	123	*9*	10	11	586	*44*
8	1200	500	*42*	26	*2*	24	27	24	673	*56*	90	19		220		Broken in Centrifuge			
9	1133	255	*22*	42	*4*	31	11	8	443	*39*	165	8	1	348	*31*	5	6	312	*27*
10	1033	363	*35*	35	*3*	28	24	20	641	*62*	359	7	0	734	*72*	24	16	1093	*106*
11	1033	185	*18*	1	*0.1*	26	19	25	633	*61*	38	8	6	104	*10*	7	7	386	*37*
12	933	256	*27*	0	*0*	27	11	15	475	*51*	1	0	0	2	*.2*	11	10	571	*61*
13	933	136	*15*	4	*0.4*	11	17	12	366	*39*	45	7	3	111	*12*	9	8	466	*50*
14	867	105	*12*	15	*2*	19	19	20	524	*60*	166	4	1	342	*39*	4	4	239	*27*
15	767	99	*13*	125	*16*	2	2	3	72	*9*	44	0	0	89	*12*	2	4	187	*24*
16	700	224	*32*	74	*11*	16	19	19	485	*69*	67	11	4	166	*24*	3	5	227	*32*
17	700	190	*27*	1	*0.1*	15	16	19	446	*64*	69	2	1	145	*21*	0	3	80	*11*
18	633	131	*21*	6	*0.9*	30	23	11	574	*90*	115	2	0	235	*37*	7	7	400	*63*
19	600	173	*29*	25	*4*	14	14	21	446	*74*	83	4	0	176	*29*	5	4	253	*42*
20	600	119	*20*	9	*1.5*	10	4	5	177	*29*	22	0	0	45	*7*	3	7	279	*46*
21	533	75	*12*	4	*0.7*	3	3	2	99	*19*	77	0	0	155	*29*	0	1	27	*-5*
22	500	102	*20*	49	*10*	12	9	14	315	*63*	90	53	3	294	*59*	1	2	93	*19*
23	500	66	*13*	18	*4*	9	7	5	191	*38*	30	1	0	63	*13*	1	4	133	*27*
24	467	182	*39*	30	*6*	25	15	14	489	*105*	49	9	4	126	*27*	6	4	293	*63*
25	433	88	*20*	0	*0*	10	11	13	307	*71*	3	1	1	12	3	6	2	227	*52*
26	367	47		0		9	10	9	259		86	.5	0	173		4	2	173	
27	367	43		0		3	8	5	152		18	2	.5	43		2	1	80	

28	367	54		2		5	9	7	187		7	1	0	16		4	1	146
29	333	110		38		11	9	9	259		10	6	0	33		7	8	413
30	333	44		82		7	7	8	199		19	11	.5	62		5	7	333
31	333	78		12		9	8	8	232		44	1	0	90		1	1	53
32	267	20		4		1	2	0	27		12	1	0	26		0	0	0
33	267	42		0		0	2	2	37		3	2		10		1	4	147
34	133	55		7		6	8	9	209		72	1	0	146		5	.5	160
35	133	13		0		1	3	0	40		11	0	0	23		0	1	27
36	67	98		12		2	5	4	107		32	1	0	67		2	3	147
37	67	49		0		3	2	4	85		19	2	1	45		3	6	240
38	67	71		0		8	2	2	112		3	.5	0	8		6	6	320
39	67	6		0		2	0	0	18		0	0	0	0		.5	0	13
40	67	23		0		0	0	1	9		0	0	0	0		.5	0	13
41	33	10		15		0	0	0	0		2	0	0	5		0	0	0
42	33	4		4		1	0	0	9		1	0	0	3		1	1	53
43	0	105		32		12	8	12	294		15	12	4	62		3	3	173
44	0	21		16		2	1	1	40		3	1	1	11		1	1	53
45	0	6		1		0	0	0	0		3	0		6		0	0	0
46	0	14		0		0	1	0	9		0	0	0	0		0	0	0

* Note: The counts of ova given above for each sample are an average of the counts of two observers, and are not necessarily the figures used in calculating the number of ova recovered per cc. (See methods).

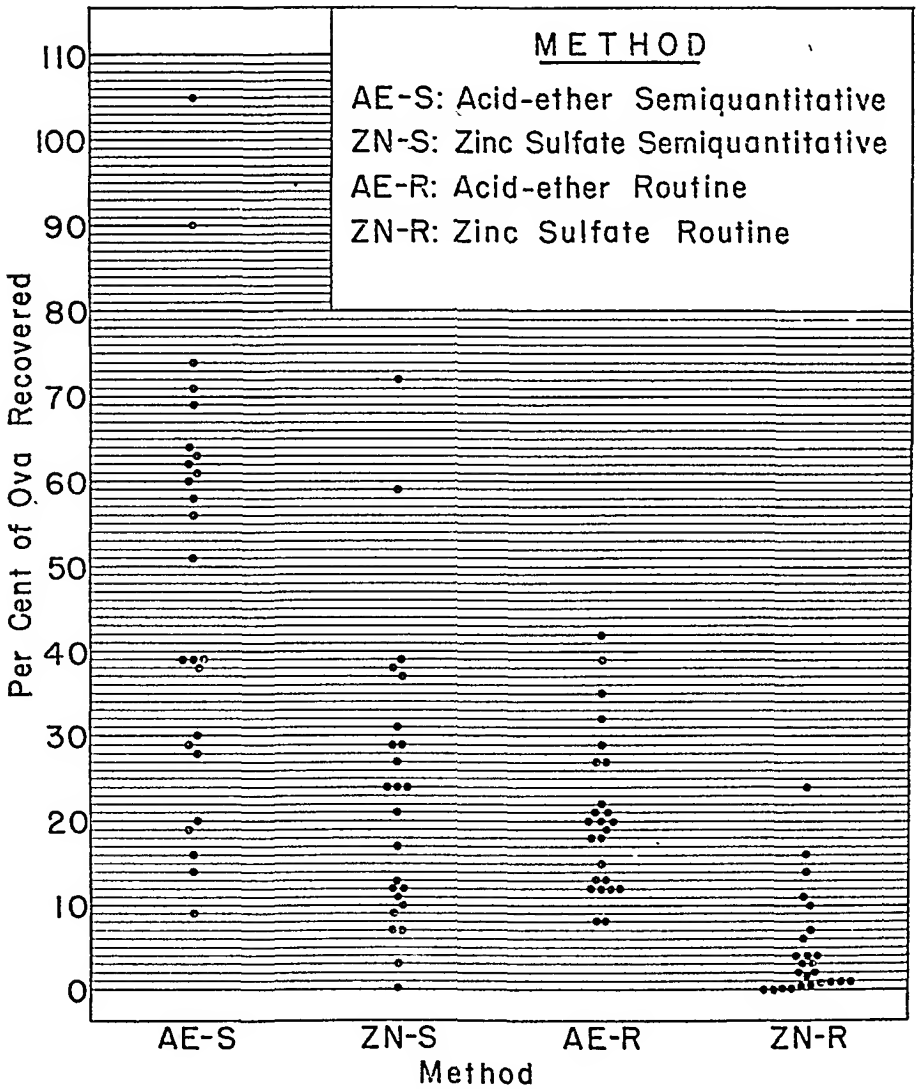

FIG. 1. Summary of recovery of schistosome ova from each of twenty-five specimens examined by the four different techniques.

preparations and could be examined in a shorter period of time.

DISCUSSION

In the present study, the routine acid-ether technique was found to be more effective in recovering schistosome eggs than was its zinc sulfate diagnostic counterpart. The semiquantitative modification of each technique was employed in an attempt to obtain information as to the relative efficiency of the two principles of concentration under conditions such as might be encountered in the course of routine survey or diagnostic work. In all probability, the yield of schistosome eggs in the zinc sulfate semiquantitative method would have been proportionately greater if a smaller volume of feces had been used, if the sediment had been stirred between centrifugations, and if more than three coverslips had been employed. However, certain specimens known to contain many eggs gave a poor yield on zinc sulfate flotation and no explanation could be discovered. It was thought that the poor yield might be due to the presence of degenerating eggs; therefore, a portion of the basic suspension of specimen #12 (which gave the lowest percentage of recovery) was sedimented in normal saline, but over 90% of the eggs appeared normal and showed active flame cell movement.

The percentage of recovery of available eggs in the series studied by the semiquantitative acid-ether method varied over a wide range, although the average recovery was 46%. While a few eggs were probably lost in the straining step (Otto et al.

1941) the variability of recovery indicated that eggs were being lost elsewhere. Examination of the debris floating at the acid-ether junction showed that it contained eggs which were mechanically entrapped by the densely packed vegetable material. This finding suggested that the addition of a detergent or wetting agent might decrease the adhesive forces holding the eggs in the debris to a point where they would sediment during the centrifugation step. Preliminary tests indicate that such is the case; the results will be reported in a future communication.

The need for a concentration technique in the search for the eggs of *S. mansoni* has been stressed by many workers. In Puerto Rico, Pons (1937) and Koppisch (1941) (1943) have emphasized the difficulty that may be encountered in finding eggs in advanced cases of schistosomiasis. The detection of light infections during surveys, while of epidemiological importance, offers similar difficulties. In the course of the survey carried out in this laboratory (Weller and Dammin, 1944) records were kept of the number of schistosome eggs recovered by the routine acid-ether technique in 1841 consecutive positive speciments; in 27% a single egg was recovered and 68% of the preparations contained a maximum of four eggs.

Sedimentation in normal saline is frequently used as a method of concentration. Yet, as noted by Hoffman et al. (1934), Scott (1937) and in this laboratory, certain types of feces do not sediment, but tend to flocculate, and in such stools sedimentation methods may fail to demonstrate eggs even though they are plentiful. In Egypt, Scott in the course of survey work found that sedimentation detected approximately as many positives as did the counting of three egg count slides made by the Stoll small drop technique, and that either method alone would detect about 85% of the positives detected by both methods together. Theoretically, the use of three small drop egg count slides will not regularly detect eggs when fewer than 67 per cc. of feces are present. From the data obtained in the present study it would appear that the acid-ether method might demonstrate concentrations of schistosome eggs that would be missed by three Stoll small drop egg count slides. It also recovers eggs from stools that flocculate when sedimented in saline or water.

The advantages of the acid-ether technique as to rapidity and simplicity of performance are apparent. Other helminth eggs and Strongyloides larvae appeared to be concentrated by the acid-ether technique as efficiently as were schistosome eggs, although no counts were done. The acid-ether method has certain inherent disadvantages. No separation can be made between live and dead eggs. At present, it does not permit an accurate estimate to be made of the number of eggs being passed. The relatively small amount of fecal material examined is a disadvantage, but is one that could be partly overcome by the selection of material from the surface of formed stools. Because of these disadvantages it is doubtful if the acid-ether method would equal in efficiency such specialized diagnostic techniques as the hatching method (Fülleborn, 1921) or the rectal swab method (Khalil and Salah el Din, 1930).

SUMMARY AND CONCLUSIONS

A uniform suspension was prepared from each of 46 fecal specimens selected because they were known to contain relatively large numbers of eggs of *Schistosome mansoni*. The number of eggs per cc. in each suspension was then determined by the Stoll dilution egg counting technique. The suspensions were examined by the routine diagnostic modification and also a semiquantitative modification of the acid-ether centrifugation technique and the zinc sulfate flotation technique and counts were made of the eggs recovered by the four methods. For twenty-five specimens found to contain over 400 eggs per cc., the results of the counts obtained by the various techniques were expressed in terms of the percentage of the available eggs that were actually recovered.

The acid-ether technique was found to be superior to the zinc sulfate flotation technique as a routine method for the isolation of schistosome eggs, recovering a greater number of eggs from 41 of the 46 specimens. In the group of 25 specimens, the acid-ether routine method gave an average percentage of 20.6% while the zinc sulfate counterpart gave an average of 4.6%. The semiquantitative modification of the acid-ether technique gave an average recovery of 48.2% while the zinc sulfate semiquantitative method gave an average recovery of 22.2%. The acid-ether preparations showed no distortion of the eggs, and usually contained less extraneous fecal debris than did the zinc sulfate preparations.

Although the acid-ether technique has inherent disadvantages the simplicity and efficiency of the method are such that it would appear to deserve further investigation as a routine diagnostic and

survey procedure for the recovery of the eggs of *Schistosoma mansoni*.[4]

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COMPARATIVE STUDIES ON ENTEROZOIC PARASITE OVA AND CYSTS CONCENTRATING PROCEDURES

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During the past 7 years the author has collected and studied from several hospitals and other sources stools known to be contaminated with enterozoic parasite cysts and ova. Initially several concentrating procedures were compared but subsequently only $ZnSO_4$ and de Rivas as the most efficient methods were used. The comparative results follow.

TABLE 1

Comparative Efficiency of Concentrating Procedures

SPECIES	DE RIVAS	$ZnSO_4$
Cysts		
Dientamoeba fragilis	−1	2
Endamoeba coli	80	32
Endamoeba histolytica	1	5
Endolimax nana	−1	4
Iodoamoeba bütschlii	1	5
Chilomastix mesnili	−1	3
Giardia lamblia	25	9
Retortomonas intestinalis	−1	1
Ova		
Diphyllobothrium latum	15	8
Dipylidium caninum	6	6
Taenia saginata	1	4
Ascaris lumbricoides	24	21
Ancylostoma duodenale	25	16
Enterobius vermicularis	−1	3
Necator americanus	20	18
Trichuris trichiuris	75	55
Fasciolopsis buski	12	9
Schistosoma mansoni	4	4
Schistosoma japonicum	10	7

Interpolated on basis of saline slide as unity.

−1 indicates that the procedure was less efficient than the control saline film.

MATERIAL AND TECHNIQUE

Recent stools were electrically mixed with a physiological saline (NaCl or Ringer's) solution until diluted adequately to filter through two layers of gauze; these filtrates, frequently agitated, were examined as a plain control film, and as $ZnSO_4$ and as de Rivas concentrate films for comparative results.

The control film was made by mixing in 1:1 ratios this filtrate and physiological saline. The cysts or ova under study were then counted per 30 microscopic fields (high dry for cysts, and low for ova) (see table 1).

The de Rivas centrifugation film was made by mixing 5 cc. of the filtrate with 30 cc.[2] of 5% acetic acid, this was again filtered, and an equal volume of ether added to the filtrate. This was shaken until it jelled,[3] then centrifuged forcibly for 10–15 minutes. The 3 upper layers (see de Rivas) were decanted leaving 2–4 drops of centrifuged sediment. Two drops of this were pipetted to a slide and the ova or cysts counted as previously.

The $ZnSO_4$ centrifugal flotation film was prepared by mixing five cc. of the same agitated filtrate with 60 cc.[2] of tap water, filtered, centrifuged at approximately 2,600 R.P.M., and then supernatant fluid decanted. This washing procedure was repeated until the supernatant fluid was clear. The washed sediment was then mixed with $ZnSO_4$ of 1.180 specific gravity and centrifuged. Two drops of the surface film containing the ova and cysts were then pipetted to a slide for examination.

RESULTS

The results are presented in table 1. These comparative figures are multiples of the saline film which was interpolated to unity. *Endamoeba coli*, for example averaged 1 cyst per 4 high dry fields in the saline film, 20 per h.p. fields with de Rivas, 8 with $ZnSO_4$. The study included 1–3 stools for each represented parasite, and 30 microscopic fields on each film.

EVALUATION

Specific: Both techniques concentrated cysts of *Endamoeba coli* and *Giardia lamblia* very well; approximating, apparently, 100% efficiency (Faust). Other cysts were better concentrated by the $ZnSO_4$ procedure (table 1).

[1] Captain, M.C., U.S.A.

[2] In some preparations indicated quantities were proportionately reduced.

[3] If the mixture does not jell, thorough shaking is indicated.

Some ova likewise were more plentiful on the de Rivas film (Table 1). The greater adequacy of collecting the concentrated ova was probably responsible for this increased efficiency in the de Rivas procedure. *Diphyllobothrium* of the Platyhelminthes and *Enterobius* of the Nematyhelminthes were apparent exceptions.[4] Usually ova of *Diphyllobothrium latum*, *Ascaris lumbricoides*, *Ancylostoma duodenale*, and *Necator americanus* were plentiful enough to be diagnosed without concentrating.

General: The disadvantages of the de Rivas procedure was that it was selective, concentrating poorly or not at all several cysts and ova (table 1); and that it produced some cytological distortion.[5]

The advantages were that it was a rapid procedure and concentrated some cysts and ova (table 1) to 2–4 drops of sediment.

The advantages of the $ZnSO_4$ procedure was that it was much less selective, concentrating more nearly uniformly all cysts and ova, and that it produced a minimum of distortion.

The disadvantages were that it required more time in the washing process. Furthermore the total film concentrate was more difficult to transfer to a microslide than was the centrifuged sediment of the de Rivas procedure.

Both procedures may be inadequate for identifying some cysts, and therefore should be supplemented by stained slides (Brown 1944).

[4] Enterobiasis is much more effectively diagnosed by perianal scrapings.

[5] Some of this can be prevented by using Schaudinn's fixative instead of 5% acetic acid.

As a routine diagnostic procedure for all ova and cysts, $ZnSO_4$ proved to be the preferred film. For concentrating appropriate ova and cysts for class room or laboratory stock the de Rivas procedure was unexcelled; likewise it was preferred when following a patient under treatment for helminth parasites.

SUMMARY

1. Concentrating procedures add nothing to specific identification but do increase survey incidence.

2. $ZNSO_4$ centrifugal flotation concentration is preferred for routine stool examinations.

3. De Rivas centrifugation concentration is preferred for selected repeat examinations and preserved stocks.

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A Manual of Tropical Medicine. By THOMAS T. MACKIE, Colonel, M.C., A.U.S., GEORGE W. HUNTER, Major, M.C., A.U.S., and C. BROOKE WORTH, Captain, M.C., A.U.S. Illustrated. Pp. i–xix, 1–727. W. B. Saunders Co., Philadelphia, Pa., 1945.

In these days, when almost every month a new work appears upon Tropical Medicine, one naturally inquires into the reason for the appearance of any new work on the subject which is already covered by many excellent treatises of recent date. The authors believe that there is need for a concise work upon the subject and the Surgeon General of the Army, Major General Norman T. Kirk states in his "Foreword" "That there has long been need for a concise treatise in this field. While a large body of authoritative information exists in textbooks, the place for a convenient manual of tropical diseases has not been filled." The reviewer, after carefully reading most of this book, agrees that it does fill the need for such a treatise and congratulates the authors upon the production of a work that should fill the needs of medical students and practicing physicians as well as of physicians serving in the Army, Navy and Marine Corps. The descriptions of the various infections and diseases are at once brief and accurate and sufficiently detailed for diagnostic purposes while the illustrations are many and excellent. The book contains much recent material that is not contained in some of the more recent texts upon tropical medicine and for this reason is most valuable.

In reading this work it should be remembered that it is not intended to be a complete scientific consideration of the subject, as, for instance, the splendid work of Stitt and Strong, so that many facts regarding the various phases of the subject are not mentioned because of the desire to present a work which would embody in as small a space as is possible all of the really essential data, a desire which has been achieved by the authors. However, one does miss at least a sentence or two upon some of the more rare conditions, as "Bullis fever" for example, which is now apparently proven to be a distinct infection, but which is not even mentioned by name in this book. This is the more surprising as the infection was first differentiated by officers of the Medical Corps of our army.

The book is beautifully printed and illustrated and may be cordially recommended as an excellent manual upon tropical medicine.

CHAS. F. CRAIG.

Penicillin Therapy, Including Tyrothricin and Other Therapy. By JOHN A. KOLMER, M.D., etc. Pages 11–302. New York, D. Appleton Century Company, 1945.

This is a timely review of the rapidly expanding literature on Penicillin and allied substances.

Chapters on "The Production of Penicillin" and "The Administration and Dosage of Penicillin" are particularly well presented. The use of the substance locally and the methods of systemic administration are described in detail.

Following the general discussion on treatment, many diseases are discussed individually as to their therapeutic response to Penicillin. These include staphylococcic septicemia, subacute bacterial endocarditis, actinomycosis, pneumonia, gonorrhea, and syphilis.

The properties and clinical application of allied antibiotics are reviewed in less detail. Tyrothricin gramicidin S., Streptothricin, Patulin and Chlorophyll are included in the discussion.

The book sets forth a good deal of practical information. It will be of value to the practicing physician who is unfamiliar with antibiotic therapy.

H. C. FISCHER.

REPORT OF AN ATTACK OF BLACKWATER FEVER SUBSEQUENT TO INDUCED MALARIA[1]

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AND

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The following account of an attack of blackwater fever is recorded partly because of the detailed history of malarial background which was available in this instance, and partly because the condition had not previously complicated any of our induced malarial infections. James, Nicol, and Shute (1932) had reported its occurrence in two patients undergoing therapy with *Plasmodium falciparum*.

HISTORY OF ANTECEDENT MALARIAL INFECTION

The patient (Mrs. P. S.) was a white woman, aged 37 years, committed to the Florida State Hospital as psychotic, with a diagnosis of cerebrospinal syphilis. The administration of malaria therapy, which had been recommended for her by the hospital medical staff, involved an infection with each of the three plasmodial species. The following summary of her malarial experience in hospital is illustrated in figures 1 and 2, which show daily parasite densities in the peripheral blood as well as the clinical response.

A vivax infection (figure 1) was first induced by the use of infected mosquitoes on July 26, 1943. It was characterized by a 15-day incubation period, 27 quotidian proxysms (a five-day remission separated the first 22 from the last five), and a spontaneous termination. The patient's peripheral blood became microscopically negative for parasites by November 18, without medication. *P. vivax* was subsequently observed in this patient's blood films on only two occasions, viz., a single parasite on January 24, and one on February 20, 1944.

[1] Presented before the joint session of the American Society of Tropical Medicine and the National Malaria Society, St. Louis, Mo., November 15, 1944.

The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of The Rockefeller Foundation, in cooperation with the Florida State Board of Health and the Florida State Hospital.

As figure 2 shows, a supplementary natural inoculation was made on March 10, 1944, with *P. falciparum*, six months after termination of the vivax attack. Characteristic, somewhat irregular

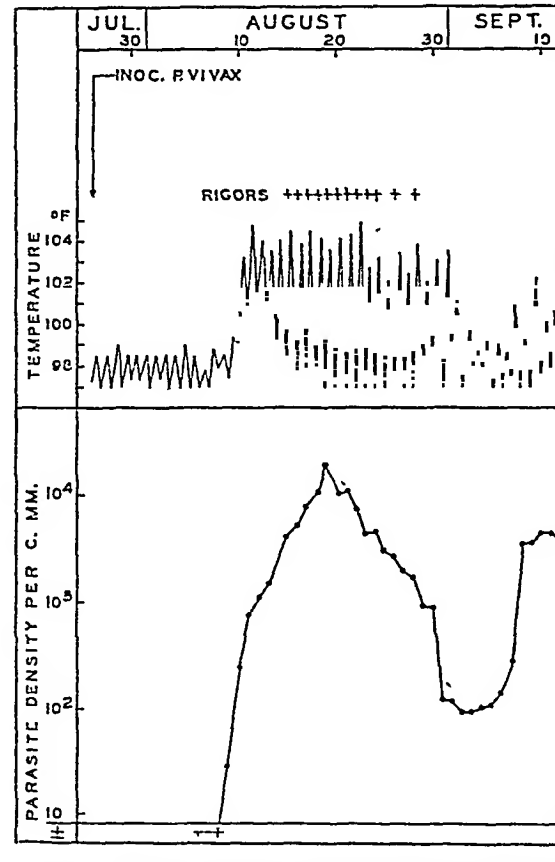

FIG. 1. Clinical response of Patient P. S. to induced *P. vivax* infection, and the daily parasite densities in the peripheral blood of the patient.

clinical activity followed a 12-day incubation period. A course of quinine was initiated April 8 and continued for 10 days (it was believed that some of the earlier doses had not been ingested). *P. falciparum* was not found in the blood films

for April 22, 23, and 27. A third infection was induced by the intravenous injection, on April 20, of blood containing *P. malariae*. Doubt concerning its probable therapeutic value, however, was occasioned by the unusually slow development of this parasite, and its failure, at densities of several hundred per cubic millimeters, to provoke clinical activity (both features are apparent in figure 2). The infection was therefore terminated by plasmochin in June. *P. malariae* was not detected in the blood films after June 16.

The persistence of *P. falciparum*, which was noteworthy and possibly significant in this case, may be traced in figure 2. The recurring parasitemic waves in May, July, and early August should be noted, together with the fact that subsequent to the middle of May, such activity provoked only minor clinical response; not more than four febrile reactions even slightly exceeded 100°F. Although daily parasite enumeration was interrupted in July and August, weekly observations (positive) were available. Thus it may be seen that *P. falciparum* was present in this patient's peripheral blood practically constantly from March 19 to the time of the attack of blackwater fever, a period of five months.

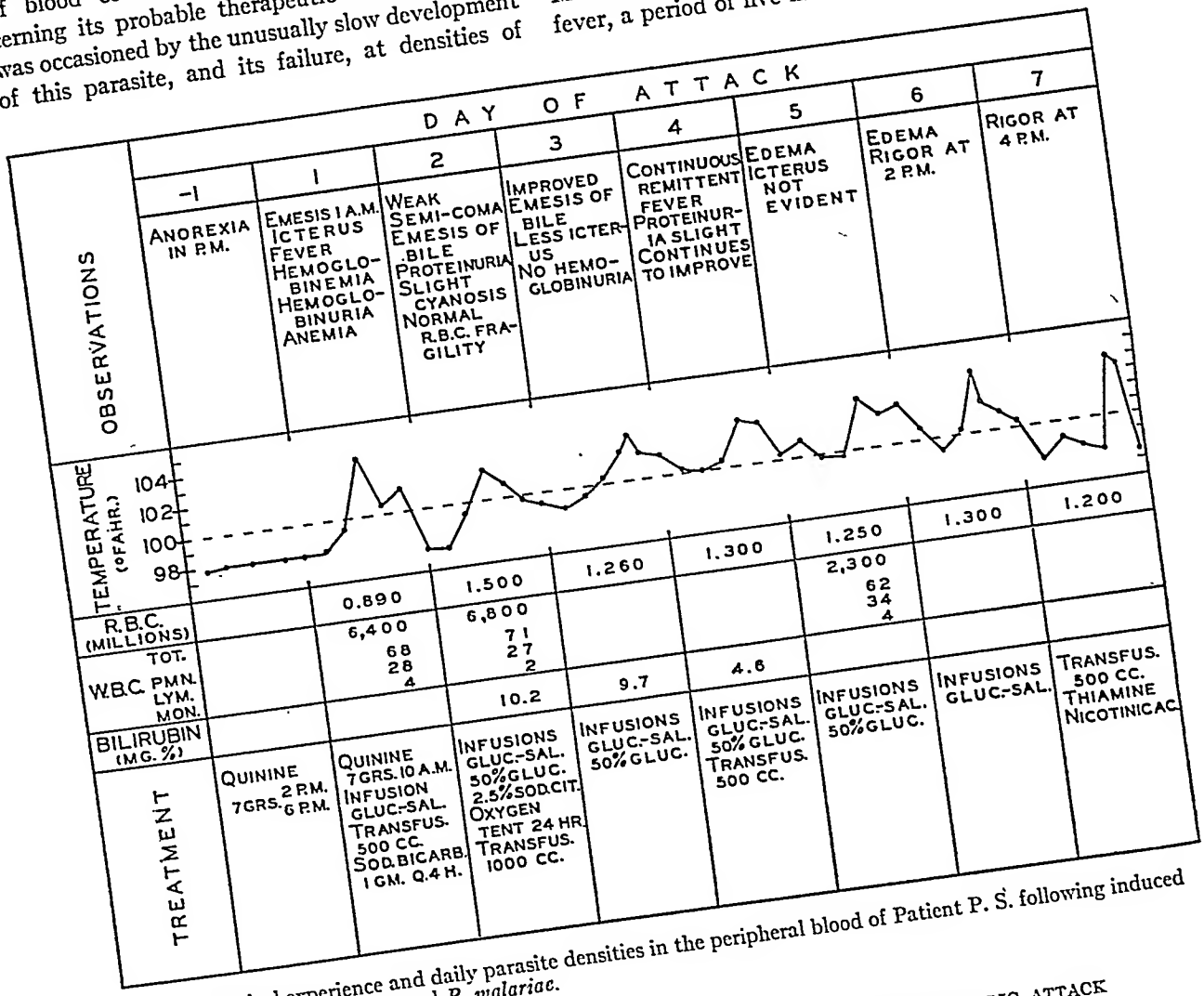

FIG. 2. Clinical experience and daily parasite densities in the peripheral blood of Patient P. S. following induced infections with *P. falciparum* and *P. malariae*.

THE HEMOGLOBINURIC ATTACK

The major clinical events concerning the hemoglobinuric episode are illustrated in figure 3.

August 21 (Day 1). On the afternoon of this day a course of quinine was initiated preparatory to returning the patient to the general wards. She received two seven-grain doses, one each at 2 and 6 p.m. She refused her evening meal but complained of nothing specifically.

August 22 (Day 1). At 1 a.m. the patient vomited some undigested food. Her temperature was normal at this time, as it also was at 4 a.m. Emesis occurred again at 7 a.m., when it was noted

that a marked degree of jaundice was present. The temperature at noon had risen to 103°F. without a preceding rigor, and by 4 p.m. it had dropped to 100.6°. Late that afternoon about seven ounces of urine of a dark reddish brown color was voided. The presence of hemolysis and a reddish brown color in the serum was observed. The erythrocyte count was found to be 890,000 per c.mm. At 9 p.m. the patient vomited considerable bile-stained material. A liquid stool contained much bile, and the skin over the entire body was intensely icteric.

August 23. On the second day the patient's pulse was weak, though regular. No improvement in the icterus was apparent. She was quite weak and very drowsy. The spleen was palpable on ordinary inspiration. Urine voided during the morning continued to be dark brownish-red. A small amount of bile-stained fluid was vomited. The erythrocyte fragility test yielded a normal result. Some cyanosis of the finger nail beds was present. Her temperature rose to 102° at noon and was the same at 2 p.m. By 6 p.m. her condition appeared somewhat improved, including the cyanosis noted earlier. During the preceding 24 hours an output of 18 ounces of urine had been measured, and a larger amount had been passed in the bed.

August 24. A morning specimen of urine was obtained, and for the first time since the afternoon of August 22 the urine was of a definitely lighter shade than noted above. The patient's blood serum was a brownish yellow in color. The blood sedimentation test showed a drop of 95 mm. in 60 minutes. In general the patient looked better and her pulse, though not strong, was regular and of fair quality. Cutaneous icterus was less marked. Her temperature had risen to 102° at 4 p.m., and to 103° at 6 p.m. It had not returned to normal by midnight. The output of urine continued to be satisfactory.

August 25. The patient's general condition appeared definitely better on the fourth day. Her erythrocyte count was 1,300,000 per c.mm. Her temperature, which had been above 100° for 24 hours, rose to 103° at 4 p.m., and at midnight was still 100.2°.

August 26. The patient continued to improve. Her urinary output for the preceding 12 hours had all been secured and it balanced the fluid intake, 900 cc. Icterus had disappeared from the skin. Her temperature by noon had fallen below 100° for the first time in 48 hours. By 4 p.m., however, it had risen to 103.4°. Slight edema of the face was noted.

August 27. The patient's fluid intake for the preceding 12 hours had been matched by the urine excreted in that period, 1,200 cc. At 1:45 p.m. she experienced a rigor for the first time during the attack. Her temperature at 2 p.m. was 102°.

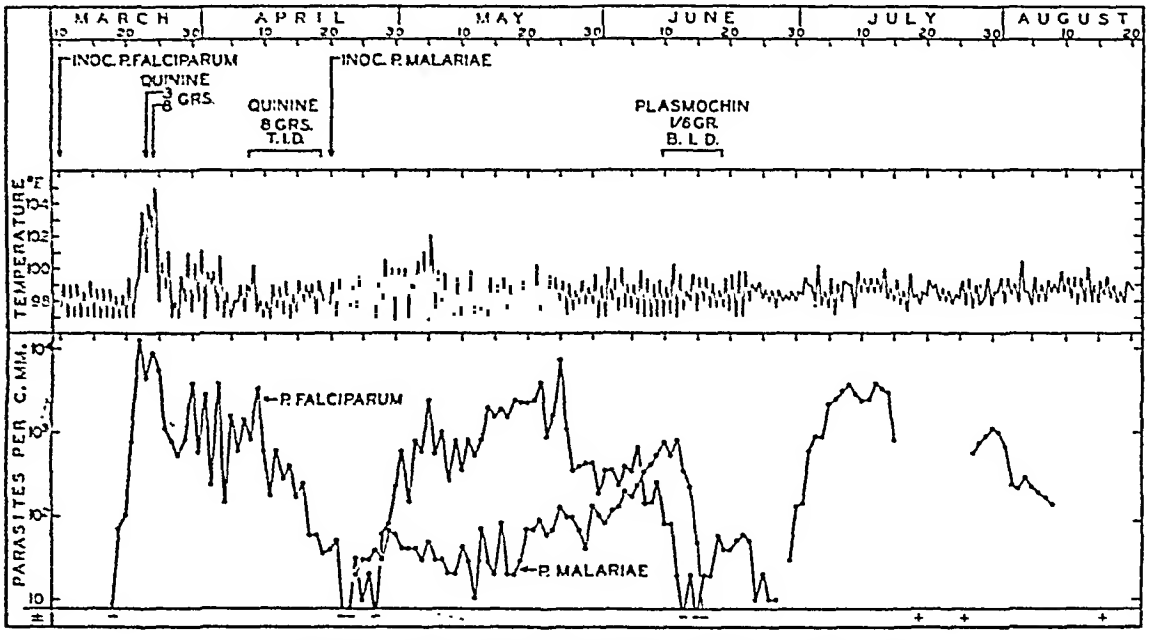

FIG. 3. Major clinical events of hemoglobinuric attack of patient P. S.

The rigor lasted about 45 minutes, and by 3 p.m. the temperature had risen to 104°. Toward the end of the afternoon her abdomen was seen to be distended, apparently with fluid, and there was some facial and pretibial edema.

August 28. The patient's temperature returned to 98.8° by 3 a.m., but dropped to 96.6° at 4 and 5 a.m. Her face was edematous. She had refused liquids during the night, and the 12-hour fluid intake (270 cc.) was about a quarter of the output (1,170 cc.). Her abdomen was less distended. Her temperature, which had remained normal during the day, was 97.8° when a rigor occurred at 4 p.m. The rigor ended at 5 p.m. on a temperature of 101.4°. At 6 p.m. the temperature had risen to 104°; it was the same at 7 p.m., then gradually returned to normal by midnight.

Treatment. As figure 3 shows, the patient was given infusions comprising 5 per cent glucose in physiological saline, and 50 per cent glucose from the first day, and on one occasion 2.5 per cent sodium citrate. Transfusions of 500 cc. of citrated blood, followed by normal saline were given on the first, second (two), fourth, and seventh days. Sodium bicarbonate was administered in one-gram doses at four-hour intervals. At noon on the second day the patient was placed in the oxygen tent for a 24-hour period. The flow of oxygen was started at seven, and reduced a few hours later to five, litres per minute. A course of thiamine chloride and nicotinic acid was commenced on the seventh day.

Convalescence. The patient apparently was safely over this hemoglobinuric attack on August 29. The edema had disappeared. Her temperature did not subsequently rise above the normal range, and no untoward symptoms were elicited on or after this date. The fluid intake and output continued to be satisfactory; urinalyses yielded normal findings and there was no evidence of renal sequelae. The chief damage to be repaired, in so far as could be determined, was the marked loss of blood, and by August 29 she appeared to be on the way to replacing that; the erythrocyte count had risen to 1,600,000 per c.mm. Two weeks later (September 11) the red cell count was 2,700,000 and the hemoglobin 7.1 grams per cent. On August 31 the icterus index was 5. On the latter date also, serum protein determinations showed the albumen and globulin values and the A/G ratio to be within normal limits.

Additional Laboratory Notes. The presence of plasmodia in blood films during attacks of blackwater fever has not been consistent, according to various reports. In the case of the episode we describe, parasites were not found from the onset of the attack until the third day after fever was last noted, when a gametocyte was observed in the thick smear on August 31. Subsequent to that date occasional gametocytes were found, and trophozoites were observed in small numbers commencing September 18. The latter forms increased in number to 450 per c.mm. on September 24, thereafter falling to very low levels.

Figure 3 shows that the total and differential leucocyte counts were within normal range on the first and second days of the attack, and that a leucopenia, with relative lymphocytosis, existed on the fifth day. Leucopenia was still present, though in lesser degree, two days after the abatement of fever. A normal leucocytic picture was noted a week following the attack.

The urine of this patient during the hemoglobinuric episode presented the characteristic appearance and contents. At the beginning it exhibited the color of port wine. Much albumen was present, and on standing, a layer of brownish-gray sediment settled out. Microscopically this was made up largely of brown-stained granular debris and cellular constituents, the latter chiefly epithelial and pus cells. Intact erythrocytes were not found. The third day specimen was definitely lighter, a brownish yellow, with no suggestion of hemoglobin in it. Excepting for the quite evident bile pigment, the urine voided on the fourth day was not remarkable; albumen could not be detected (heat test).

As figure 3 shows, the quantitative Van den Bergh test revealed 34 times the upper normal amount of serum bilirubin on the second day, and still 15 times the maximum value on the fourth day. It will be recalled that cutaneous icterus was not detected on the fifth day.

Comment. Two points in particular concerning the malarial history of this patient merit attention. Firstly, the patient had experienced three malarial infections, one with each species of plasmodium. within less than 12 months. The etiological significance of the multiple, heterogeneous malarial infections is, on the basis of our experience, questionable. It has been our practice to utilize more than one species of plasmodium when this was considered necessary in order to secure adequate therapy. Never before has hemoglobinuria followed such procedure. Furthermore, the hypothesis that certain plasmodial strains show greater

tendency to provoke hemoglobinuric attacks is not supported by the pedigree of the strains concerned in this report. The vivax strain with which this patient was infected has been propagated naturally by us since its isolation locally in 1931. The falciparum strain, also of local origin, has been in our possession four years. The quartan strain, though new to us, had been used a number of years for therapeutic purposes. None of these strains had any record of involvement in attacks of blackwater fever.

The second noteworthy feature of the malarial background was the prolonged presence of the falciparum parasite in the peripheral blood. This organism had been demonstrable in the blood smears almost continuously for the five months (and provoking a small amount of minor clinical activity during the three months) immediately preceding the hemoglobinuric episode. It may well be that persistence of the falciparum infection played a significant etiological rôle in the subsequent hemoglobinuric event. James *et al.* had encountered similar protracted quinine-resistant falciparum infections (with Sardinian and Italian strains) in their two patients who developed blackwater fever.

Of etiological interest is the relationship of quinine ingestion to the onset of this attack. There is abundant precedent for circumstantially incriminating the drug as an incitant. At least it may be stated that hemolysis probably commenced within 12 hours of the first dose of quinine. The existence of an inherent sensitivity to the drug is ruled out through failure of the patient to react adversely to the course of quinine given in April. The possibility of that medication forming a part of the etiological mechanism, however, cannot be evaluated here. We may only conclude that between that time and August 21 the one or more missing and essential parts were fitted into place.

There was no rigor to mark the onset of hemolysis in this instance, as is frequently reported. It seems probable, however, that the emesis which occurred at 1 a.m. on August 22, 11 hours after the initial dose of quinine, coincided with that process. The pronounced degree of icterus noted six hours later, 7 a.m., would support that belief.

The loss of erythrocytes within the first 24 hours of the attack was great. The red cell count on June 26, the last recorded during the two months preceding the hemoglobinuria, was 3.4 million per c.mm. Minor parasitic activity such as the patient had during that period usually takes but a small toll of erythrocytes (the acute phase of her primary attack in March reduced the red cell count only 400,000 per c.mm.). If she entered the blackwater fever attack with a density of 3.0 to 3.5 million erythrocytes per c.mm., as seems not unreasonable, she lost almost three-quarters of her circulating red cells in less than 24 hours. The rapid development of a marked degree of jaundice and the observation, even on the second day, of 34 times the upper normal value for serum bilirubin lend credence to the extensive destruction.

Since there is no specific treatment for blackwater fever, therapy was directed chiefly toward a partial replacement of blood loss by transfusion, and maintenance of an adequate fluid intake and acid-base equilibrium by infusion with glucose and saline. A very moderate amount of sodium bicarbonate was given by mouth. In all, five half-liter transfusions of citrated blood were given during the first five days. In spite of these, the patient's erythrocytes did not exceed 1.3 million during the seven days following the onset. Obviously her hemopoietic mechanism did not function significantly in that period, for we had no reason to believe that there was any appreciable hemolysis after the second day.

Contrary to a former theory, acidosis is not generally present in blackwater fever; and even the hypothesis that oliguria and anuria are the result of blockage of the renal tubules by hemoglobin degradation products, precipitated in an acid urine, and cellular debris, is open to doubt. Added to which, the fact that acid urine may be excreted although an alkalemia be present, and that in such cases administration of alkalis not only may not alter the reaction but are likely to be distinctly harmful, contraindicate the indiscriminate use of intensive alkaline therapy in this condition. As Foy *et al.* (1943) and Maegraith (1944) indicate, alkalis, while desirable in acidosis, may nevertheless upset kidney function in the presence of dehydration and salt deficiency, both of which may obtain in blackwater fever. Fairley (1944) believes that alkaline therapy should be controlled by alkali reserve determinations. Wakeman (1932) suggested that the rational way to meet the salt and fluid loss was through the parenteral administration of saline and glucose. He felt that early treatment by transfusion and infusion might prevent suppression, minimize impairment of renal function, and thereby eliminate the later secondary effects of the disease.

Possibly the relatively early and generous ad-

ministration of blood, saline, and glucose to our patient prevented the development of anuria. The presence of edema from the fifth to seventh days of the attack may have meant that we gave too much fluid, or sodium chloride, under the circumstances. Her general condition on the morning of the second day had been obviously unsatisfactory, perhaps partly due to anoxemia and partly to shock. We do not know whether the additional oxygen she received during part of the second and third days was of real value, although she appeared to benefit from it.

Occasional falciparum parasites were still demonstrable in this patient's blood films in December 1944. Preparatory to a full course of quinine, she was given smaller doses of the drug as follows: December 19, two grains; December 28, five grains; January 8, 1945, three doses of seven grains each. None of these induced detectable hemolysis.

SUMMARY

The malarial background and clinical aspects of an attack of blackwater fever have been described; the episode occurred in a white female patient, age 37, subsequent to malaria therapy.

The patient had experienced an infection with one strain of each plasmodial species during the preceding 13 months; *P. vivax* had been allowed to disappear spontaneously, and *P. malariae* had been suppressed by a course of plasmochin; the falciparum infection, in spite of a course of quinine administered following the acute phase of the attack, persisted for five months, with minor parasitic and consequent clinical activity during the latter period of the infection.

Onset of the hemoglobinuric episode probably occurred within 12 hours of initiation of quinine medication; it was characterized by emesis and the rapid development of icterus; fever, unaccompanied by rigor, appeared about 11 hours after onset.

The attack lasted one week and was featured by: *anemia*, involving the loss of 70 to 75 per cent of circulating erythrocytes in the first 24 hours; moderate *emesis*, chiefly bilious early in the attack; daily *fever* in the afternoons (continuously at or above 100°F. for 48 hours on the third to fifth days), with rigor on the sixth and seventh days; *hemoglobinemia*, *hemoglobinuria*, and *proteinuria* during the first two days; *bilirubinemia* (10.2 mgm. per cent on the second day) and intense *icterus* which gradually decreased after the second day; a state of *near collapse* on the second day; *edema* on the fifth to seventh days; the absence of microscopically detectable parasites from the peripheral blood.

Treatment chiefly comprised blood transfusions, infusions of saline and glucose, moderate dosage of alkali by mouth, and supplementary oxygen.

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THE DOUROUCOULI (AOTUS) IN LABORATORY CYCLES OF YELLOW FEVER[1]

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From the Villavicencio Field Laboratory, Villavicencio, Colombia

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The douroucouli is of particular interest in relation to the epidemiology of jungle yellow fever because it is said to be the only monkey to be found in certain parts of Colombia where yellow fever is apparently endemic in the forests. In a recent article Boshell and Osorno (1944), writing of the Muzo region, state: "It has been conclusively established by observations over several years that there are no monkeys in this region with the exception of small groups of *Aotus lanius*." The animal has not been studied in the laboratory except for one specimen reported on by Davis (1931). This was bitten by *Aedes aegypti* infected with the Asibi strain of yellow fever virus. It showed no temperature reaction, but blood serum taken a month after its exposure to the mosquitoes protected a rhesus monkey against inoculation with a test virus dose. Apparently no attempt was made to recover circulating virus. Recent work has made it clear that the reactions of American monkeys to the African Asibi strain of virus may be very different from their reactions to local strains (Laemmert, 1944). The apparent resistance of this single aotus tested by Davis thus cannot be given much weight.

We have recently started to re-examine the susceptibility of various neotropical mammals to infection with the virus of yellow fever by the method of attempting to interpose them in laboratory cycles of local virus strains, using as a standard the saimiri-haemagogus cycle (Bates and Roca, 1945). The douroucouli was among the first animals so tested, and the results indicate such a high order of susceptibility that, in view of the special importance of the animal, separate publication seems warranted. The techniques used in the maintenance and study of the mosquito-mammal cycles have been fully described in the previous article.

Dr. G. H. H. Tate of the American Museum of Natural History kindly examined one of our experimental animals; he writes that this and other specimens from Villavicencio may be referred to *Aotus trivirgatus*. The nomenclature of the genus *Aotus* is in a confused state (for references, see Tate, 1939) and many names have been proposed for what are probably a few rather closely similar geographical populations. There are two recognizable forms in Colombia, though their taxonomic status is uncertain: a mountain form with very long, lax fur, for which the oldest name is *lemurinus*, and the short-furred lowland type, *trivirgatus*. Dr. Tate writes us that he is unable to find obvious or consistent cranial differences between these two forms, and it seems unlikely that the two are specifically distinct. Whether such geographically defined populations would differ in susceptibility to yellow fever virus is a question on which we as yet have no data. The origin and use of the vernacular name "douroucouli" is discussed by Simpson (1941). We include a photograph of one of the Villavicencio animals (fig. 1).

FIG. 1. THE DOUROUCOULI (AOTUS TRIVIRGATUS)

We are greatly indebted to Dr. Jorge Boshell-Manrique for giving us free access to some of his unpublished epidemiological studies, which seem to

[1] The studies and observations on which this paper is based were conducted with the support and under the auspices of the Section of Special Studies maintained by the Colombian Government and the International Health Division of The Rockefeller Foundation.

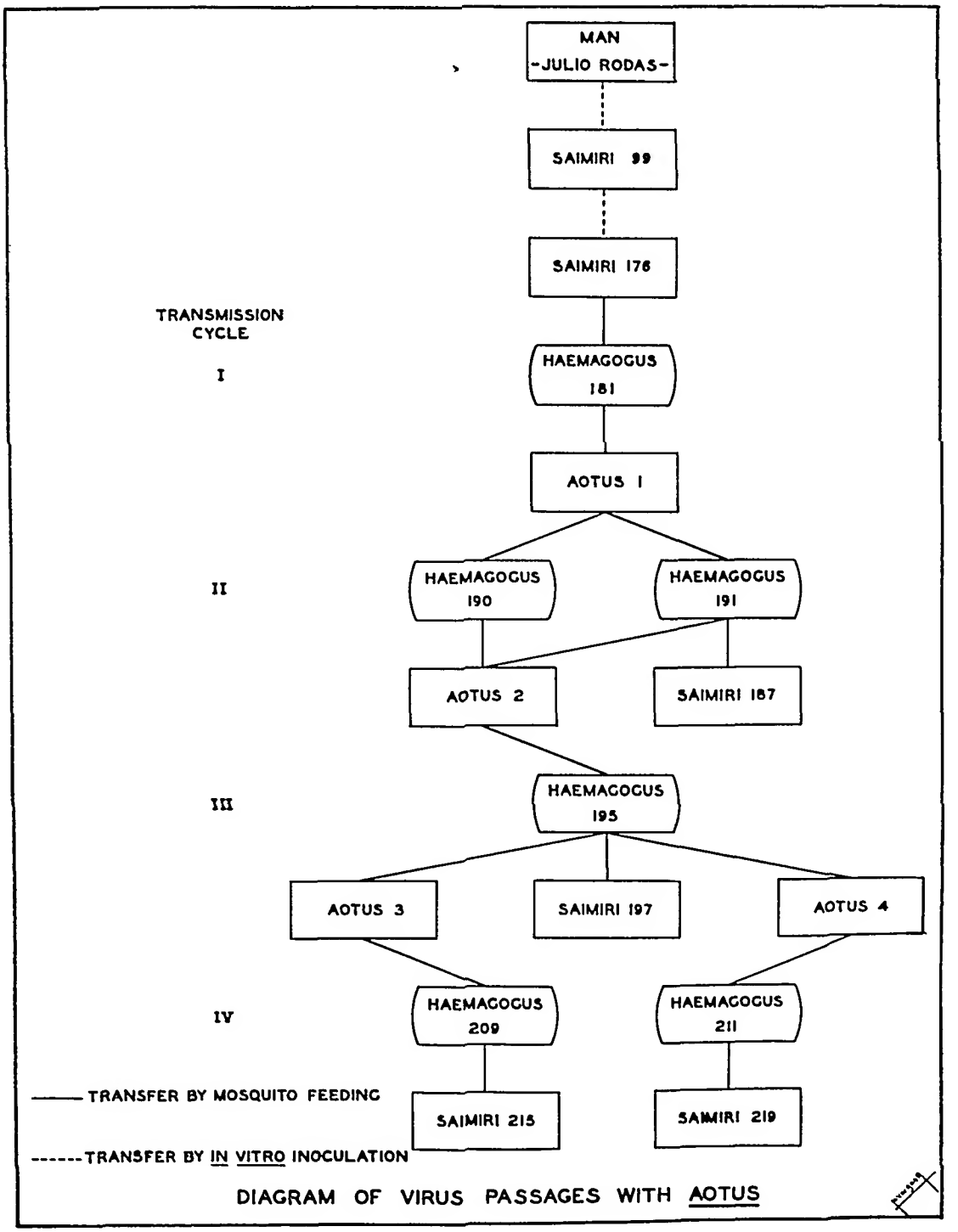

FIG 2

implicate the douroucouli in natural virus cycles; and to Dr. Augusto Gast-Galvis for making pathological examinations of the liver tissue of animals dying in the course of infection.

MAINTENANCE OF CYCLES

Four douroucoulis have been interposed in laboratory virus cycles, as shown in the diagram (fig. 2).

Each of these has shown a sharp febrile reaction and a very high titer of circulating virus, and has died on the fourth or fifth day after infection, with signs characteristic of fatal yellow fever. Virus has been carried from saimiri to douroucouli, douroucouli to douroucouli, and douroucouli to saimiri, by the bite of *Haemagogus capricornii*, without the slightest difficulty. The virus strain used was from a patient (Julio Rodas) who had a non-fatal case of yellow fever. It was isolated by the inoculation of the human serum in a saimiri monkey. A series of saimiri-haemagogus cycles was maintained with this virus similar to those described for Perez virus (Bates and Roca, 1945) with identical results; in particular, the percentage of haemagogus showing virus under given environmental circumstances was the same with the two strains. Rodas virus has caused fatal infections in saimiris from the beginning. We have used it in place of Perez virus because of its more recent isolation, and because it has never been passaged through mouse brain and was only once desiccated (between saimiris 99 and 176; see diagram). We are attempting to maintain this strain by constant mammal-mosquito passage, thus avoiding any further "unnatural" manipulation.

The characteristics of infection in *Haemagogus capricornii* after feeding on douroucoulis differ considerably from those observed in mosquitoes infected on saimiris. We think that this illustrates the overwhelming importance of the "dosage factor" in mosquito infections, since the mosquitoes pick up much more virus from douroucoulis than from saimiris. The phenomenon of different infection rate for mosquitoes kept at different temperatures, described in our report on saimiri-haemagogus experiments (Bates and Roca, 1945), is not apparent in mosquitoes infected on douroucoulis. Virus has been recovered from practically all of the haemagogus that fed on douroucoulis, regardless of the environmental temperature. (Tests were made in a range between 20°C. and 30°C., as in the saimiri experiments.) Haemagogus infected on douroucoulis are able to transmit by bite to douroucoulis, saimiris, or baby mice 9 and 10 days after the infectious meal if kept at 30°C., as compared with the minimum 13 day period found in similar experiments with saimiris. The maintenance of virus with the use of douroucouli monkeys, *Haemagogus capricornii* mosquitoes, and Rodas virus seems to be as easy as maintenance with rhesus, *Aedes aegypti*, and Asibi virus. The mosquitoes even feed more readily on douroucoulis than they do on saimiris—a phenomenon for which we have no ready explanation.

CHARACTERISTICS OF INFECTION IN DOUROUCOULIS

The histories of the four douroucoulis used in infection experiments are summarized in table 1. Each animal showed a febrile reaction. Normal temperatures are similar to those of saimiris (Bates, 1944), varying between 38° and 39°C. Animals 1, 3, and 4 all had abundant altered blood in the stomach at autopsy. Dr. Augusto Gast-Galvis has kindly given us the following notes on the results of histological examinations of liver material:

"There is an acidophilic coagulative necrosis with a salt and pepper distribution throughout the entire lobule, but most intense in the mid-zone. There are abundant Councilman bodies with well-defined characteristics—acidophilia, well-defined border, fatty inclusions, and nuclear chromatolysis. Intranuclear inclusions are also present. There is a fatty infiltration, with droplets of small and medium size, and in some cases one may observe hepatic cells impregnated with bile pigment."

The animals were bled and tested for circulating virus by the intracerebral inoculation of serum in white mice on the third day and daily thereafter. We have pointed out (Bates and Roca, 1945) that mouse mortality with these local "pantropic" virus strains may be very irregular, which makes the calculation of titers by the usual method undependable. Baby mice (seven days old) seem to be uniformly susceptible on intracerebral inoculation, but it is difficult to maintain a small mouse colony in such a way as to provide adequate numbers of baby mice of uniform age for extensive titrations. We made several titrations with aotus sera, but the titer proved to be so fantastically high that in no instance did we reach the end point. Intracerebral inoculation of 7-day-old mice with serum of aotus 4 taken on the fourth day, killed 3 of 4 mice when a dilution of $1:10^8$ was used, and 2 of 4 mice when the dilution was $1:10^9$. It seems probable from the incomplete titrations that all four animals showed titers of the order of magnitude of $1:10^9$. The results of one titration by parallel inoculation of mice of different age groups and saimiri monkeys are given in table 2, since this series shows nicely the difference in susceptibility exhibited by mice of different age groups. This titration was made with rehydrated serum.

Our method of desiccating serum is not very efficient and rehydrated serum almost always shows a considerable loss of virus titer; it is thus not unreasonable to suppose that the original virus titer for baby mice in the case of this animal was also of the order of magnitude of $1:10^9$. Since the maximum titer of virus circulated by saimiris is usually about $1:10^6$, this may well mean that there is a thousand times more virus in circulation in an infected aotus than in an infected saimiri.

TABLE 1

Histories of aotus monkeys infected with yellow fever virus

AOTUS NO.	HISTORY OF MOSQUITOES			HISTORY OF MONKEY							
	Lot no.	No. biting	Days after infectious meal	Circulating virus: day			Fever		Day of death	Stomach haemorrhage	Liver lesions
				3	4	5	Day of onset	maximum temperature			
1	181	5	16	+	+	+	3	40.1	5	yes	yellow fever
2	190	8	17	+	+		2	40.0	4	no	yellow fever
3	195	10	10	+	+		3	40.5	4	yes	yellow fever
4	195	2	17	+	+		3	40.5	5	yes	yellow fever

TABLE 2

Titration of rehydrated serum of aotus 1, fourth day after infection

(Mice inoculated intracerebrally with 0.03 cc., saimiri monkeys intraperitoneally with the same amount of the corresponding dilutions.)

DILUTION	MICE: 7 DAYS OLD		MICE: 21 DAYS OLD		MICE: 70 DAYS OLD		SAIMIRI MONKEYS						
	Mortality	A.S.T.*	Mortality	A.S.T.	Mortality	A.S.T.	No.	Circulating virus: day					Day of death
								3	4	5	6	7	
1:10	4/4	6.8	6/6	8.3	4/6	13.5							
$1:10^2$	5/5	7.6	6/6	9.7	5/6	12.3							
$1:10^3$	5/5	7.0	5/6	11.5	1/6	19.2							
$1:10^4$	5/5	7.8	3/6	14.6	2/6	18.7	189	+	+	+			6
$1:10^5$	5/5	8.2	3/6	17.5	2/5	17.2	192	+	+	+			6
$1:10^6$	5/5	8.8	2/6	18.4	4/6	17.2	193	+	+				5
$1:10^7$	4/5	11.8	2/6	18.8	0/6	20.0	194	+	+	+	+	+	survived

* A.S.T. = average survival time, calculated on a basis of 20 days of observation.

DISCUSSION

A large population of douroucoulis would clearly provide a fertile field for the development of jungle yellow fever. Unfortunately, because of the nocturnal habits of these monkeys, we have very little idea as to how abundant they may be in different areas. Dr. Jorge Boshell-Manrique has recently been making studies of the relative abundance of various mammals in an area in Colombia called Volcanes, where during 1943 he recovered virus with astonishing frequency from *Haemagogus capricornii*. Douroucoulis seem not to be particularly abundant in this area, and during the first year he succeeded in catching only three, though others were seen. All three animals, however, showed clearly positive protection tests for yellow fever. These were the only primates that have so far been found resident in the area, and almost the only mammals that have given positive protection tests for yellow fever. Such studies are necessarily made after an epidemic, and there is no way of knowing whether an explosive outbreak of yellow fever, such as characterized this Volcanes area, might have been preceded by a relatively large population of a highly susceptible animal such as *Aotus*. From the laboratory results, it seems remarkable that any of the animals would have survived.

Knowledge of the habits of douroucoulis is

largely limited to casual references by the traveller-naturalists. The observations of Enders (1935) on the Panamanian species are particularly interesting in connection with the possible rôle of the animal in yellow fever. He writes: "The Night Monkey is strictly nocturnal . . . and is the only monkey on Barro Colorado Island that appears to have a definite sleeping place. This home is usually a hole high up in some tree or in a dense tangle of vines in the tree tops. Nor do they move about in groups larger than family groups. These habits make them difficult to see unless one is familiar with their nesting sites. As would be expected from a knowledge of their habit of living in holes in trees, they are limited in distribution to the more mature forest where such cavities are found. Here families were found living within a hundred meters of each other which may indicate a greater density of population than actually exists. Nevertheless they cannot be called rare."

The habit of spending the day sleeping in the canopy zone of mature forest would certainly expose these animals to haemagogus mosquitoes; and, as pointed out above, this monkey makes an ideal source of blood meals for haemagogus in the laboratory. In view of the fact that *Aotus* occurs in yellow fever areas where other susceptible primates seem to be absent, it must be given careful consideration in any study of the epizoology of the disease.

SUMMARY

Four douroucoulis (*Aotus trivirgatus*) from the Villavicencio area of eastern Colombia were tested in laboratory cycles of yellow fever with the mosquito *Haemagogus capricornii* and a local strain of virus. All four animals showed acute, fatal infections, characterized by fever and a very high titer of circulating virus; death occurred on the fourth or fifth day after infection. Three of the animals showed stomach hemorrhage, and liver tissue from all four showed lesions characteristic of fatal yellow fever in man and rhesus monkeys. Virus was transmitted by the bite of the mosquito *Haemagogus capricornii* from saimiri to douroucouli, douroucouli to douroucouli, and douroucouli to saimiri. There is some evidence that the douroucouli may be important in the epidemiology of yellow fever, since it is said to be the only monkey in certain areas in Colombia where the disease is endemic.

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SPECIFICITY OF THE TOXIC FACTORS ASSOCIATED WITH THE EPIDEMIC AND THE MURINE STRAINS OF TYPHUS RICKETTSIAE[1]

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Immunological studies on epidemic and murine strains of the rickettsiae of typhus fever, carried on over a period of years, have shown that the two agents are closely related, in that animals which recover from infection with one strain are immune to the other (1). However, Plotz (2) has shown that the complement-fixing antibodies produced by the two types of rickettsiae are highly specific when purified rickettsiae are used as antigens. A new method of investigating the specificity of typhus strains was afforded by the observations of Gildemeister and Haagen (3) that a "toxin" is associated with living murine rickettsiae and that this "toxin" could be neutralized by sera from convalescent epidemic and endemic human cases. Bengtson, Topping, and Henderson (4), and Henderson and Topping (5), greatly extended the work on this toxic factor. They showed that a "toxic substance" was present in yolk sac suspensions of epidemic rickettsiae and described methods for standardizing it and for estimating the amount of neutralizing antibodies in immune sera. It should be noted that the term "toxin" employed by Gildemeister and Haagen, and the term "toxic substance" used by Bengtson, Topping, and Henderson refer to the same thing. Since the lethal factor is intimately associated with living rickettsiae, and its true nature has not been established, we shall use the general term, "toxic factor," or "toxin" (in quotes), for the purpose of this report. The present report presents the results of studies on the specificity of the toxic factors associated with the rickettsiae of epidemic and murine typhus.

METHOD

Rickettsial "toxins" were obtained from yolk sacs infected with two epidemic (Breinl and Madrid) and two murine (Wilmington and Castaneda) strains of typhus. The infected sacs were selected and processed according to the general method described by Henderson and Topping (5). However, titrations were all made by using the intraperitoneal route of inoculation, as employed by Gildemeister and Haagen (3) and Craigie (6). To titrate the "toxin," serial two-fold dilutions of the stock suspension were made with Ringer's solution; 1 cc. of 1:10 normal human or guinea pig serum was then added to 1 cc. of each of the dilutions of "toxin." The mixtures were shaken well, incubated at 37.5°C. for 30 minutes, and 0.2 cc. amounts were injected intraperitoneally into 4–8 white mice weighing approximately 15 grams. One minimum lethal dose (M.L.D.) was considered to be present in the dilution containing that fraction of an infectious yolk sac which was capable of killing 50% of the injected mice. This dilution was usually a yolk sac suspension of 1:10 by weight for epidemic "toxin" and 1:20 for murine "toxin," except for the Madrid strain,[3] which was even more toxic than either of the murine strains.

The titration of neutralizing antibodies in immune sera was carried out as follows: 0.5 cc. quantities of serial two-fold dilutions of serum were mixed with 0.5 cc. amounts of toxic suspension which had been diluted so that each 0.2 cc. of the final serum-"toxin" mixture contained 2 M.L.D.'s. A standard neutralizing serum was not included with each protocol, but was used with different pools of toxic material in order to make sure that the selected 2 M.L.D.'s were neutralized to the same titer. However, a control normal serum was included in every series of serum titrations, in order to be sure that the toxic factor had not lost potency. After incubation at 37.5°C. for 30 minutes, 0.2 cc. amounts of serum-"toxin" mixture were injected intraperitoneally into each of 4 mice. The time at which death of the animals occurred was carefully recorded during the succeeding 5 hours, with a final recording at 24 hours. Preliminary experiments, carried out in conjunction with Lt. E. J. Bell, Sn.C., indicate that the neutralizing antibody titers obtained by the intra-

[1] Report to the Director of the United States of America Typhus Commission, 27 September 1943.

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[3] (An epidemic strain.)

peritoneal method, just described, appear to be comparable with those obtained on the same sera when tested by the intravenous technique of Henderson and Topping.

The titers of neutralizing antibodies presented in this report represent the dilution of serum before toxic material was added.[4]

It was naturally of interest to compare the neutralizing titers with the titers of complement-fixing antibodies in the same serum specimens. The author is indebted to Lt. K. Wertman, Sn.C., for the performance of the complement fixation tests which have been included in the following text and tables.

PRELIMINARY STUDIES WITH THE EPIDEMIC (BREINL) TOXIC FACTOR

At the outset, it was of interest to learn whether neutralizing antibodies might be present in sera of vaccinated persons who had failed to develop demonstrable complement-fixing antibodies. The results obtained with five such sera are shown in table 1: It is obvious that the titers of neutralizing antibodies are very low. As Henderson and Topping have pointed out (5), these titers would be of little or no significance were it not for the fact that serum from these same individuals taken before vaccination failed to show any evidence of neutralizing antibodies. The appearance, therefore, of even low titers is thought to be significant. All five individuals had some neutralizing antibody, though in only three of them (C. Z., L. C., and R. R.) was it in a titer of 1:3 or better. None of them showed complement-fixing antibodies at 1:3.

TABLE 1

Comparison of complement fixing- with epidemic neutralizing-antibody titers in sera of post-vaccine and convalescent cases

SUBJECT	VACCINE§	AMOUNT OF VACCINE	TIME OF BLEEDING	COMPLEMENT FIXATION EPIDEMIC ANTIGEN	NEUTRALIZATION EPIDEMIC "TOXIN"
C. Z.	VE-2A whole epi.	2.25 cc.	18 days	0*	1:3†
J. S.	Craigie 13-2 epi-end.	4.6 cc.	14 days	0	1:1†
N. R.	Craigie 13-2 epi-end.	5.0 cc.	20 days	0	1:2†
L. C.	E-62 (whole epi.)	1.5 cc.	13 days	0	1:8†
	Craigie 1 3-2epi-end.	2.0 cc.			
R. R.	E-62 (whole epi.)	1.5 cc.	13 days	0	1:8†
	Craigie 13-2 epi-end.	2.0 cc.			
F. S. #1	Brill's Disease—Convalescent		12th day of disease	1:384	1:320 (p)‡
F. S. #2	Brill's Disease—Convalescent		22nd day of disease	1:192	1:160
J. M.	Brill's Disease—Convalescent		3 yrs. 48 days	1:12	1:5
R. W.	Brill's Disease—Convalescent		1 yr. 143 days	1:12	1:5
G.p. #382	Breinl Convalescent guinea pig			1:384	1:10
G.p. pool	Breinl Convalescent guinea pigs			1:12	1:4

* Lowest dilution of serum used in the complement fixation test was 1:3.

† Sera of these individuals, obtained before vaccination, showed no neutralizing antibodies, undiluted (1:1).

‡ p = partial protection.

§ The vaccine was a 10% yolk sac suspension which had been ether-extracted. It was usually given in 0.25–100 cc. doses, subcutaneously, at weekly intervals.

Table 1 also contains comparative complement-fixing and neutralizing antibody titers on several sera from humans recovered from Brill's disease as well as guinea pigs recovered from infection with the Breinl strain of rickettsiae. It is of interest to note the relatively close correlation between the titers observed in the two tests, with the exception of G. p. 382. These observations are supplemented, not only for the epidemic strain but also for murine typhus, by data to be presented in table 2. These observations correspond, in general, with the preliminary data recorded by

[4] All titers, therefore, represent initial dilutions of serum.

Bengtson, Topping, and Henderson (4). The occasional failure to find correlation between the levels of the two antibodies suggests that complement-fixing and neutralizing antibodies may be separate entities.

SPECIFICITY OF EPIDEMIC AND MURINE TOXIC FACTORS

In initial experiments, "toxin" was prepared from both the epidemic (Breinl) and murine (Wilmington) strains of typhus and tested with sera of guinea pigs convalescing from infection with Breinl, Wilmington, or Castaneda strains of typhus. In these tests, undiluted serum from animals which had recovered from infection with either of the three strains was capable of neutralizing both epidemic and murine "toxins." Similar results were reported by Gildemeister and Haagen (3), using murine "toxin" and epidemic sera diluted 1:2. Subsequently, when quantitative titrations of neutralizing antibodies in human sera were made, differences were noted in the titers obtained with epidemic and murine "toxins." Samples of sera from patients with epidemic typhus were chosen which showed a high titer in complement fixation tests with epidemic antigen, but which had a low titer or were negative when tested with murine antigen. Similar criteria were used in the selection of murine sera. Each of these sera was titrated for neutralizing antibodies, using toxic material from four different strains (Breinl, Madrid, Wilmington and Castaneda). The results obtained in the investigation of 12 such cases are shown in table 2.

The six sera from cases of epidemic typhus all showed high titers of antibodies capable of neutralizing both strains of epidemic "toxin," the titration endpoints ranging from 1:80 to 1:640. However, these same sera had relatively low titers when the two murine "toxins" were used, *viz.*, from 1:5 to 1:20. Though to a lesser degree, the sera from the six cases of endemic typhus show a higher neutralizing capacity to the homologous than to the heterologous toxic factor. Despite the lower degree of specificity with murine sera, the data are sufficiently clear-cut to indicate that the toxic factors of epidemic and murine typhus and their respective antibodies are distinct entities. The individuality of the neutralizing antibodies was investigated further by means of absorption studies.

TABLE 2

Estimations of neutralizing antibodies against epidemic and murine toxic factors in sera of typhus patients

CASE	COMPLEMENT FIXATION		NEUTRALIZATION			
	Epidemic antigen	Murine antigen	Epidemic toxic factor		Murine toxic factor	
			Breinl	Madrid	Wilmington	Castaneda
Epidemic Typhus						
Bis-Mexico—41	1:800	1:10	1:640	1:640	1:5 (p)*	1:5
Guatemala-Tecpan #3	1:384	1:12	1:80	1:320	1:10	1:10
Mexico—42X	1:640	1:10	1:160	1:320	1:40	1:40
Human—875	1:640	1:10	1:160	1:640	1:10	1:10
Ecuador—#1	1:640	1:10	1:320	1:640	1:5	1:5
Ecuador—#12	1:640	0	1:320	1:640	1:5 (p)	1:10
Endemic Typhus						
Low	1:10	1:160	1:20	1:20	1:40	1:40
Kee	1:24	1:384	1:40 (p)	1:20	1:80	1:80
Pet	1:10	1:160	1:10	1:20	1:40	1:80
Le H	1:12	1:192	0 (<1:5)	0 (<1:5)	1:40	1:80
Cros.-2	1:10	1:160	1:5 (or less)	0 (<1:5)	1:40	1:40
Sym.-3	1:20	1:320	1:10 (or less)		1:40	

* p = partial protection.

ABSORPTION OF NEUTRALIZING ANTIBODY

Purified and washed suspensions of epidemic (Breinl) and murine (Wilmington) rickettsiae of known nitrogen content were used for the absorption of antisera by the procedure previously de-

scribed (2). The rickettsiae were washed twice, immediately before using, with potassium phosphate buffer (pH = 6.2; ionic strength = 0.2). Identical quantities of each strain of rickettsiae (as indicated by equal N-content: 2 mg./cc.) were then separately mixed with equal volumes of an undiluted epidemic typhus serum, and similarly, with two portions of a murine serum. The mixtures were placed in an icebox and shaken occasionally to resuspend the rickettsiae. After standing overnight, the tubes were shaken again and centrifuged at 10,000 r.p.m. in the cold (0°C.) for 1 hour to sediment all rickettsiae. The clear supernatant sera were removed and tested for neutralizing and complement-fixing antibodies.[5] Table 3 summarizes the results which were obtained:

TABLE 3

Specificity of neutralizing antibodies as shown by absorption of sera

IMMUNE SERA	NEUTRALIZING TITER BEFORE ABSORPTION		ABSORBING ANTIGEN	TOXIC FACTOR USED	NEUTRALIZING TITER AFTER ABSORPTION	
	Epidemic	Murine			EPIDEMIC	MURINE
Ecuador #12 (epidemic typhus)	1:160 (c.f. = 1:640)	1:5	Epidemic rickettsial antigen	Epidemic	1:40 (c.f. = 1:320)	
			Murine rickettsial antigen	Epidemic	1:160 (c. f. = 1:640)	
Sym. #3 (murine typhus)	1:10 (or less)	1:40 (c.f. = 1:320	Epidemic rickettsial antigen	Murine		1:40 (c.f. = 1:320)
			Murine rickettsial antigen	Murine		1:5 (c.f. = 1:160)

It will be noted that when the epidemic serum (Ecuador #12) was absorbed with epidemic rickettsiae, the epidemic neutralizing titer was reduced from 1:160 to 1:40, whereas absorption with the same quantity of murine rickettsiae (2 mg./cc.) caused no detectable reduction in epidemic neutralizing antibodies. The results with the murine serum (Sym. #3) were even more striking, for absorption with epidemic antigen produced practically no reduction in murine neutralizing capacity, while absorption with murine rickettsiae reduced the titer from 1:40 to 1:5. In each case, absorption with the homologous antigen reduced the complement-fixing antibody titer (c.f.) by one dilution, whereas absorption with the heterologous antigen did not alter the original titer. Thus, the results of absorption studies add further evidence for the belief that the toxic factors of epidemic and murine typhus are antigenically distinct.

[5] It should be noted that the amount of absorbing antigen used in this experiment was sufficient only to carry out a partial absorption of antibodies from the sera. A small quantity of antigen (2 mg./cc.) was purposely selected so that any possible differences would be apparent in the *relative* absorptions of the homologous and heterologous antibodies. Subsequent experiments by Plotz and Snyder have shown that both the heterologous and homologous antibodies can be removed in certain definite proportions by the absorption of sera with large amounts of antigen (to be published).

DISCUSSION

The foregoing experiments indicate a marked separation between the epidemic and murine toxic factors, and between their corresponding antibodies, thus providing another example of antigenic differences in the rickettsiae of epidemic and murine typhus. They also demonstrate that there is some overlap with low dilutions of serum. This parallels the findings of Plotz (2), who has previously described the presence of specific complement-fixing antibodies induced by infection with the two organisms.

The existence of two major toxic factors is clearly shown by the use of rickettsiae from different sources. Neutralizing titers against the "toxin" from the Castaneda murine strain are similar to those obtained on the same sera with

Wilmington murine "toxin." Likewise, the neutralizing titers obtained on each kind of serum are of comparable magnitudes when either Breinl or Madrid epidemic "toxins" are used. From these data (table 2) it is seen that consistent differences in neutralizing titer have not been obtained when a given serum is tested with different strains of the *same type* of "toxin." However, within the epidemic and murine categories there are strain differences in potency of the toxic factors (*e.g.*, Madrid epidemic is more toxic than Breinl epidemic). The important difference, which proves specificity, is obtained when different types of "toxin" are used on the same serum.

CONCLUSIONS

1. The toxic factors associated with living murine and epidemic typhus rickettsiae and the antibodies which neutralize them appear to be immunologically distinct.

2. A comparison of the titers of complement-fixing antibodies and neutralizing antibodies in individual immune sera shows considerable correation. However, the occasional lack of close correlation suggests that the antibodies may not be the same.

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DENGUE-LIKE FEVER ON THE ISTHMUS OF PANAMA

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From September 1941 through March 1942 an acute illness, unusual by local criteria, appeared on the Panama Canal Zone. The following description of this disease was determined from a study of thirty-two white adult patients admitted to a medical service of Gorgas Hospital.

INCIDENCE, EPIDEMIOLOGY

These cases represent but a few of the total number and although the exact number of cases which occurred on the Isthmus is not known, the incidence was great enough to be considered mildly epidemic.

A history of known or probable contact with others similarly affected was established in over one-half of all cases. Three and four cases respectively originated from the same domicile. Although isolation precautions were not employed in the hospital cases, only three cases occurred in the professional personnel and only one of these had had known previous contact with the condition. Most of the patients had not traveled into the unsanitated jungle areas for a considerable period of time previous to the onset of their illness. A consistent history suggestive of a transmission vector was not obtained, although in some cases a history of having been bitten by mosquitoes previous to the onset of their illness was elicited. The significance of this finding was impossible to evaluate since it was not established in the majority of cases. In those cases which gave such a history, the interval between the time of having been bitten and the onset of the illness was obscure. The patient's ability to accurately differentiate between mosquito and other insect bites raises a serious element of doubt, and no supporting epidemiological field investigations were done.

There was no apparent relationship between the incidence of the disease and season. On the Isthmus of Panama the seasonal variations are essentially dependent upon the presence or absence of rainfall. The "wet season" lasts from May to December with the remaining months constituting the "dry season." Although two-thirds of all cases occurred during the transition months of December and January, the other cases first started to appear in the "wet season" and continued to appear in the "dry season." Therefore, no etiological relationship was determined as to the seasonal incidence of the disease.

There was no demonstrable relationship between the incidence of the disease and the local population distribution. Age and sex groupings, exclusive of the pediatric group which was not observed in this study, showed no relationship between the incidence or the degree of severity.

SIGNS AND SYMPTOMS

The onset, which was sudden in one-half of the cases, was frequently marked by a frank chill. In the remainder, however, malaise, aches, and anorexia preceded the fever by twelve hours to four days. The fever rose rapidly after onset, at times reaching its peak for the entire illness within the first few hours. After forty-eight hours, in typical cases, there was a definite but usually incomplete remission. In those cases in which the temperature returned to normal it remained so for only a brief interval. This period was followed in one or two days by an exacerbation which terminated by rapid lysis at the tend of the fifth day (120th hour). The intensiy of the fever during the exacerbation was ordinarily not so marked as that during the initial period but this was not invariably true. The resulting fever curve showed a "saddle-back" configuration (fig. 1). In the more severely ill this pattern was replaced by a continuous spiking fever which, nevertheless, was of the same five day duration as in the milder cases (fig. 2). Of twenty-one patients, who were observed for sufficient time to determine the character of the febrile course, thirteen presented the "saddle-back" pattern. Since only those patients whose illness was most severe sought hospitalization it is possible that this does not represent an accurate picture. The peak intensity of the fever in these twenty-one cases ranged from 101° to 105°, with fifteen presenting a maximum of 102°

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to 103°. Of thirty cases, in which the duration of the fever was ascertained, twenty-two lasted 110–130 hours; two 96–110 hours; five 140–150 hours; and one terminated after 90 hours without a second temperature rise.

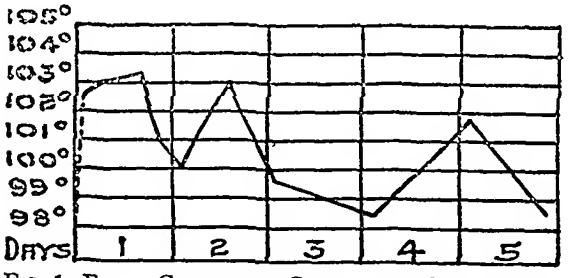

FIG. 1. FEVER CHART OF A CASE WITH A SADDLE-BACK FEVER
-----Interval between onset and admission to hospital.

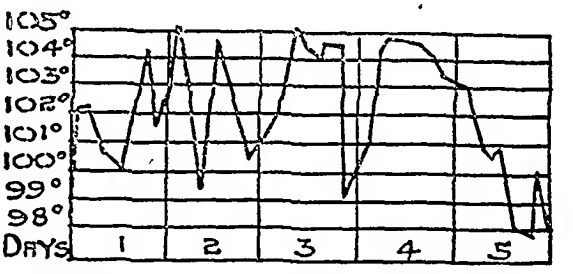

FIG. 2. FEVER CHART OF A CASE WITH A CONTINUOUS SPIKING FEVER
----Interval between onset and admission to hospital.

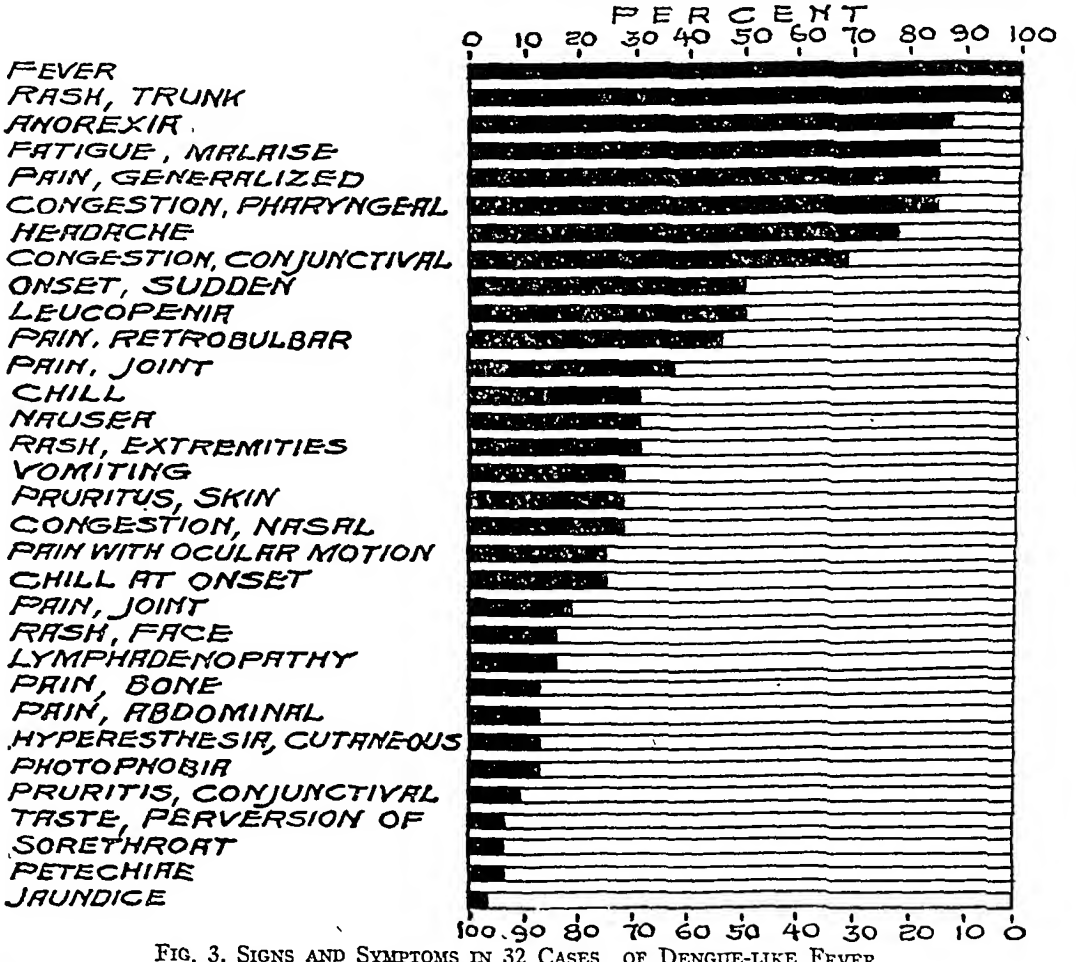

FIG. 3. SIGNS AND SYMPTOMS IN 32 CASES OF DENGUE-LIKE FEVER
■ Sign or symptom present.
□ Sign or symptom absent.

As characteristic as the fever course was the parallel variation in the intensity of the complaints. During the period of fever remission the patients frequently claimed a state of normalcy only to be disillusioned by a return of their complaints when the fever rose again. However, the complaints rapidly subsided with the final resolution of the fever. The most outstanding complaints (fig. 3) were malaise, fatigue, generalized aching, headache,

retrobulbar pain, joint pains, chills, anorexia, nausea, and vomiting. The fatigue varied from mild tiredness to severe exhaustion and frequently persisted as a postfebrile sequela lasting from several days to weeks. The body aches, which were described as muscular stiffness and soreness aggravated by motion, also varied widely in degree of severity. In many cases there was specific pain localized in the bones, joints, and back, but in only one instance was it of the severity described as "break-bone." Ordinarily generalized, in a few cases the headaches were localized in the fronto-occipital areas. Retrobulbar pain frequently appeared during the second day and was usually accompanied by moderate pain incident to ocular motion. This, when present, was so characteristic as to be considered almost pathognomonic. Transient cutaneous hyperesthesia, which manifested itself with one exception during the first day, was mild in degree. Mild to moderate pruritis appeared, with two exceptions, during the fifth to the seventh day. This was usually generalized, but occasionally it was limited to the extremities, and it never lasted for more than twenty-four hours. Mild itching of the conjunctivas, photophobia, and slight sore throat occasionally occurred during the first days of the illness. The gastro-intestinal complaints ranged from mild anorexia to protracted nausea and vomiting. The abdominal pain was never noteworthy as to location, duration, or intensity.

All the patients presented a rash. Since some were not observed at the onset of their illness it was not possible to determine in all cases when the eruption began. In twelve patients the rash appeared during the first two days and persisted throughout the disease; in ten cases, which were admitted on or after the third day, it was present on admission; while in eight it was not demonstrated until the fourth day, which corresponded to the time of the febrile exacerbation. The rash first appeared over the trunk, but in one-third of the cases it also spread to the extremities after one to three days. The face was involved in five instances; two initially, two by spread, and one simultaneously with the appearance over the trunk. Although originally ill-defined, within twenty-four hours it assumed a characteristic blotchy net-like configuration. It was a macular eruption ranging in color from pink to deep scarlet. In a few cases, however, it became frankly morbilliform. In the cases where the rash appeared early, an increase in intensity was frequently noted coincident with the febrile exacerbation. This was not interpreted as evidence of a new or different eruption. Otherwise, the character of the rash remained static after the first day. The duration varied from three to nine days with the majority of cases not fading until the seventh to the ninth days. No desquamation was noted during the postfebrile period. However, observation during this interval was frequently interrupted by early discharge from the hospital.

Petechiae and jaundice appeared among the more seriously ill patients. The petechiae, which occurred in two cases, appeared over the antecubital fossae, feet, and ankles on the fourth and seventh days respectively. Jaundice, which appeared in the most seriously ill patient of the group, was mild in degree and was not clinically apparent until the fifth day. It rapidly subsided and was thought to be the result of a transitory hepatitis.

The nasopharyngeal and conjunctival pathology consisted of only mild to moderate injection, which was in no way characteristic. Lymphadenopathy was neither marked nor generalized. The enlargement of the cervical, occipital, axillary, and inguinal nodes which were involved singly or in groups disappeared by the fifth day. There was no splenic enlargement.

The erythrocyte count and hemoglobin values were normal. The leukocyte count varied from 2000 to 8000 per cu. mm. Eleven of the cases had a leukopenia of 4500 per cu. mm. or less. In the majority of instances this appeared on the third or fourth day. A relative lymphocytosis of more than 40 per cent was found in sixteen cases during the period of leukopenia. The urine was normal and there was no increase in the sedimentation rate. Cultures of blood, urine, feces, and the Weil-Felix reaction were done in a few cases with negative results.

DISCUSSION

In 1912 Beverly and Lynn (1) reported sixty cases of dengue from the Isthmus of Panama. This report stated that dengue had been noted previously in this area in 1904. Their description was similar to the one noted here but the incidence and degree of lymphadenopathy was more marked. They made no reference to a biphasic fever or of the complaints showing a characteristic corresponding variation in intensity. A total of one-

hundred and four cases of dengue have been reported by the hospitals of the Panama Canal from 1907 through 1941 (3). Of these, eighty-three cases were first reported in 1912. The remainder were distributed sporadically with no more than six cases being reported in any one year. No cases have been reported since 1924. Deeks (2) reported a six-day fever from this region in 1914 which has been classified as dengue-like (5). The absence of joint pains, lymphocytosis, and the presence of splenomegaly with polymorphonuclear leukocytosis separate the six-day fever from the condition reported here. Additional contrast is afforded by the fact that the rash in the fever described by Deeks was petechial and did not appear until one to two days after the temperature had returned to normal.

Dengue has been clinically defined as a disease characterized by "... a very sudden onset; severe pains in the muscles, bones, and joints; marked leukopenia; a rapidly rising temperature, followed by a recession on the third or fourth day, a secondary rise, and a critical fall to normal or below normal on the fifth, sixth, or seventh day, a typical skin eruption usually appearing during the secondary rise in temperature or just before the crisis of the fever; and by marked prostration, both mental and physical (4)." The similarity between this definition and the entity described in this paper is apparent, but some of its characteristics were at variance with those which have been described as typical for dengue. There was no apparent division of the associated rash into a "primary" and "secondary" eruption. The initial eruption of dengue occurs at the onset of the fever and is said to be evanescent in nature (4). Most of the cases observed in this series were not seen during the early hours of their illness and this was judged to be the probable explanation for not having noted a typical dengue initial rash. However, the second or characteristic rash in dengue does not appear until the fever remission and usually not until the secondary fever rise (5). In these cases the eruption, which persisted throughout the illness, appeared during the first two days in twelve cases. If this eruption is to be considered the initial rash of dengue, then in over one-third of the cases the first eruption either merged with the second in such a manner that they could not be separated or the terminal eruption did not appear and the initial one was prolonged. This was the situation observed by Siler (7) in 19 per cent of experimental dengue cases. The terminal eruption of dengue is said to first appear over the hands and feet and spread in a centripetal manner, but in the cases described here the eruption appeared over the extremities in only one-third of the cases, and the spread was definitely from trunk to extremity. Bradycardia, which has been described as occurring during the terminal days of the fever and convalescence in dengue (5, 6) was not observed. Lymphadenopathy has been reported as being prominent in dengue (7). In sixty cases of experimental dengue Simmons (8) observed cervical and inguinal lymphadenopathy in forty and twenty-one cases respectively, while in this group of thirty-two cases only five developed slight lymph node involvement. Craig (4), however, states that in his experience the lymph nodes in dengue were not enlarged, although he notes that other authorities had observed enlargement.

The condition described here presents some variations from the characteristics which have been considered typical of dengue. Yet both Manson-Bahr (6) and Craig (4) make mention of the variability of the symptomatology of dengue as reported in various epidemics and in different degrees of severity. The differentiation between dengue and dengue-like fevers is difficult and must remain an open issue until such time as clinical observations are supplemented by laboratory investigations.

SUMMARY

1. A dengue-like fever occurring on the Isthmus of Panama during 1941 and 1942 is described.

2. Some of its characteristics are compared with those definitive for dengue and other local febrile entities.

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THE CHEMOTHERAPY OF HUMAN FILARIASIS BY THE ADMINISTRATION OF NEOSTIBOSAN[1]

Second Report

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Although filariasis of the bancroftian type has proved to be a much less serious problem to the military than it appeared likely to become soon after the beginning of World War II, it remains an ever-present infection in a considerable percentage of the native population of tropical areas. Because of the frequency with which serious symptoms or complications are seen in those native persons who harbor the infection, methods of controlling or eradicating filariasis have long been greatly needed. Unfortunately, the preventive measures used so successfully, by and large, by the military for the protection of its personnel would hardly suffice for stamping out the disease from civilian communities, in part because of the difficulty in rigorously enforcing such measures among civilians, as would be required over a long period. The fact, too, that among the native groups one has not only to prevent new infections but also to control or eliminate those which have been long-established adds to the general complexity. A significant step toward resolving the problem would follow the discovery of specific means whereby every infected individual in a community could be freed of the disease. Progress in this direction has been reported in recent years by several groups of investigators, usually through the administration of antimony-containing drugs (1, 2). During April and May, 1944, the authors treated in Puerto Rico, West Indies, a number of filaria-infected patients with neostibosan, a compound of pentavalent antimony. One report, from data obtained through six months after the cessation of treatment, has already appeared from the work (3). The present paper offers data on the same group of persons from observations through twelve months from the end of treatment.

[1] The work described in this paper was done in part under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the College of Physicians and Surgeons, Columbia University.

GENERAL PROCEDURES

The procedures and methods followed have already been described in detail in the first report of this work (3), but will be reviewed here briefly.

The patients.—All of the patients were native Puerto Ricans infected with *Wuchereria bancrofti.* Those less than 18 years of age (see tables) were students at insular homes for children near San Juan. Those 18 years old or more were outpatients of University Hospital at the School of Tropical Medicine in San Juan. All were free of symptoms of filariasis except one individual (No. 13, table 1), who had had periodic chyluria for several years. All the thirty treated patients (except No. 13, who was hospitalized), as well as the fifteen in the untreated control group, were engaged in their usual activities as students or as workmen throughout the period of treatment.

The administration of drug.—During the first week of treatment, all patients were given three injections of neostibosan on alternate days, the first usually containing 50 mg., the second 100 or 150 mg., and the third 300 mg. of drug. Thereafter 300 mg. of drug were given in single doses daily or on alternate days until treatment ended in 33 to 48 days. All injections were given intravenously.

The estimation of microfilariae.—The number of parasites in 60 c.mm. of nocturnal finger blood was determined before treatment began, at frequent intervals during the course of treatment, and every month thereafter for a period of one year—except in twelve persons. Observations were stopped in twelve of the patients after nine months, these persons, in whom the first course of therapy had been apparently ineffectual, being then subjected to a second course of treatment. Similarly, nocturnal blood samples (60 c.mm.) were obtained at intervals over a period of fourteen months from the fifteen untreated control patients. The blood samples were let dry on microscope slides, then dehemoglobinized in water, and stained with Bul-

lard's hematoxylin. The total number of parasites in each sample was determined by searching the entire film under 100× magnification.

the 12 remaining treated patients, who were observed for only 9 months, microfilariae declined by the ninth month from 4 to 74 per cent compared

TABLE 1

Microfilaria counts in thirty patients with filariasis (Wuchereria bancrofti) treated with neostibosan

CASE NO.*	AGE	SEX	WEIGHT	GRAMS OF DRUG GIVEN	INTERVAL OF TREATMENT	NUMBER OF MICROFILARIAE IN 60 C.MM. BLOOD FROM TREATED PATIENTS AT DESIGNATED TIMES						MICROFILARIA LEVEL, % CHANGE, ENTIRE PERIOD OF OBSERVATION
						Before treatment	At end of treatment	Months after end of treatment				
								2.5	6.0	9.0	12.0	
			lbs.		*days*							
1 LET.....	11	F	60	7.2	40	3	0	0	0	0	0	−100
2 MR.....	8	F	50	4.6	33	9	0	0	0	0	0	−100
3 VR.....	10	F	48	5.8	33	24	6	0	0	0	0	−100
4 AEB.....	16	F	106	7.2	40	15	36	8	0	0	0	−100
5 RT.....	14	F	100	7.2	40	41	36	9	0	0	0	−100
6 DG.....	12	M	73	7.5	39	21	51	6	0	0	0	−100
7 JT.....	17	M	134	8.1	39	27	42	27	0	0	0	−100
8 CIR.....	13	F	102	7.2	40	216	204	141	6	0	0	−100
9 EMD....	18	M	138	10.4	47	18	42	21	2	0	0	−100
10 MSC.....	26	M	112	10.5	47	150	120	66	10	0	0	−100
11 GM.....	15	M	114	8.1	39	33	15	15	10	1	0	−100
12 JG.....	13	M	79	8.1	39	255	204	57	14	3	0	−100
13 CP.....	21	M	125	7.6	48	15	61	—	1	2	0	−100
14 CAR.....	15	F	93	6.8	40	231	207	81	21	4	1	−99
15 JOA.....	20	M	133	9.2	54	82	8	91	52	13	1	−98
16 OA.....	21	M	146	7.2	38	36	12	0	1	1	1	−97
17 JL.....	13	M	76	7.5	39	297	294	171	111	28	18	−93
18 PB.....	11	M	58	6.9	39	177	96	114	111	104	22	−87
19 CD.....	16	F	112	7.1	40	120	126	72	42	31	†	−74
20 JR.....	16	F	85	6.0	40	154	129	138	81	69	†	−55
21 FG.....	13	M	71	8.1	39	630	624	345	217	284	†	−54
22 ME.....	16	F	102	6.9	40	136	126	111	49	65	†	−52
23 MN.....	12	F	74	6.5	40	27	54	18	12	13	†	−51
24 CF.....	8	F	52	6.4	33	123	93	90	109	65	†	−47
25 IO. .. .	14	F	138	7.2	40	129	156	84	87	71	†	−44
26 GG.. . .	14	F	140	7.0	40	216	255	180	199	139	†	−35
27 VG	16	F	118	6.2	33	54	55	66	121	47	†	−12
28 HRR.....	11	M	56	7.3	33	18	9	9	15	16	†	−11
29 DR.....	13	M	76	8.1	40	72	120	72	39	67	†	−6
30 BM.....	14	F	91	7.1	40	78	87	90	56	75	†	−4

* All the patients presented in this table were also presented in a previous report (3) after six months of observation except No. 15 who is substituted for No. 15 of the earlier paper, who left the institution in which the work was done and is no longer available.

† These patients were retreated after observation for 9 months following the initial course of drug.[2]

RESULTS

Twelve months from the end of the period of treatment, 13 of the 30 treated patients had apparently lost all their microfilariae and 5 had lost between 87 and 99 per cent of the microfilariae which were present before treatment began. In

to the number present before treatment.[2] The data on all 30 treated patients are given in table 1.

[2] After observation for the period of 9 months, these 12 patients were retreated, drug being given more intensively than during the initial course of therapy. A number of these patients are now negative for micro-

Of the control group, 3 of 15 patients presented decreases of from 31 to 80 per cent in the microfilaria level during 14 months, and 12 presented increases in this level ranging from 3 to 1200 per cent. The data on the 15 control patients are shown in table 2.

None of the patients was significantly affected adversely by the treatment. About half the 30 treated persons exhibited occasional nausea or emesis, especially in the first week or ten days of treatment. Four subjects presented low-grade fevers later in the course of therapy. In no patient, however, was it necessary to discontinue the drug, although occasional injections were omitted. In the 12 months since treatment ended, no patient has exhibited any symptom whatsoever which could be interpreted as an untoward effect of the treatment.

TABLE 2

Microfilaria counts in fifteen untreated control patients with filariasis (Wuchereria bancrofti)

CASE NO.*	AGE	WEIGHT	NUMBER OF MICROFILARIAE IN 60 C.MM. OF BLOOD AT DESIGNATED TIMES						MICROFILARIA LEVEL, PERCENTAGE CHANGE, ENTIRE PERIOD OF OBSERVATION
			First examination	After 1 month	After 6 months	After 9 months	After 12 months	After 14 months	
		lbs.							
1 FN.....	13	85	45	21	27	45	15	9	−80
2 SR.....	14	124	63	3	22	53	37	23	−63
3 MR.....	14	82	360	353	318	496	568	248	−31
4 VMO.....	12	90	309		333	343	239	319	+3
5 LS.....	14	69	135	153	162	202	143	140	+3
6 LT.....	17	115	27		31	82	53	37	+37
7 VG.....	12	78	27	31	36	73	32	40	+47
8 MR.....	12	90	315	285	333	656	297	472	+49
9 GM.....	14	96	36	60	58	92	51	69	+91
10 JM.....	9	57	87		176	270	369	198	+127
11 JF.....	16	120	3	6	6	19	13	7	+133
12 FV.....	12	63	6	6	9	7	9	17	+183
13 VB.....	14	102	9	6	13	31	15	29	+222
14 FV.....	14	96	27	31	55	83	133	90	+233
15 JR.....	8	56	3		15	37	34	39	+1200

* All are males.

DISCUSSION

One of the most significant points shown by the data of table 1 is the tardiness with which the effect of treatment with neostibosan became apparent in filariasis. By the end of the course of therapy, little or no change had occurred in the microfilaria levels of patients. Two and one-half months later, however, about 75 per cent of the treated patients showed a small but definite decrease in the number of circulating parasites. As more time elapsed, microfilaria levels either continued to decline or were maintained. After 12 months, 13 patients had apparently lost all microfilariae and 5 others gave promise of soon doing so. On the other hand, 12 patients (40 per cent of those treated) exhibited after 9 months—for reasons thus far not apparent—only small likelihood of ever losing all microfilariae as the result of the initial course of drug.

The simplest explanation for this tardy loss of microfilariae is that the neostibosan acts, not directly on the microfilariae, but chiefly on the adult parasites. It would seem that, as a result of the death of the adult worms, the production of embryos ceases; the number of microfilariae in the blood then gradually declines, therefore, since those circulating embryos which are being lost are not replaced. Evidence for this circumstance following chemotherapy of filariasis in an experimental animal has already been presented (4).

It is also significant that in the case of no patient have symptoms suggestive of early elephan-

filariae and all have shown a substantial decline in the number of circulating parasites. The data following the second course of therapy in these patients will be presented in a separate communication.

tiasis appeared following treatment. Some years ago O'Connor (5) suggested that elephantiasis results from an inflammatory response by the host to the presence of dead filarial worms. Largely following this thought, some present authorities in filariasis have questioned the wisdom of chemotherapy in this disease, fearing that by killing adult parasites, one would provoke the identical serious symptoms of elephantiasis which it is hoped to prevent through treatment. The thirteen patients negative by treatment in the present series, if followed for some years, may make possible an adequate evaluation of the essential danger, if any, in the chemotherapy of this disease.

In the earlier report on these patients (3), the authors cautioned that the effect of treatment might be distinctly temporary and that, with passage of time, the infection might relapse and microfilariae reappear in the blood of the seven patients who had become negative at that time. However, none of these seven patients has shown microfilariae at any time in the additional six months of observation. Two individuals of the present group of thirteen negative patients have now been negative for microfilariae for twelve months—i.e., since treatment ended. It seems quite likely that reappearance of microfilariae at some future time, at least in the two patients just referred to, could be accounted for only on the basis of a reinfection of these patients. Evidence is apparently accumulating from this work to indicate that once the microfilariae have disappeared from patients following treatment with neostibosan, the filarial infection is entirely eradicated and is not subject to relapse.

SUMMARY

Thirty patients infected with *Wuchereria bancrofti* were treated with neostibosan for from 33 to 48 days. After twelve months, 13 of the patients were free of circulating microfilariae and 5 others had lost from 87 to 99 per cent of their embryos, compared with the number in the blood before treatment. The twelve remaining patients showed little promise of eventual eradication of their infections after observation for nine months.

Of 15 control untreated patients infected with *Wuchereria bancrofti*, all remained infected for 14 months of observation. In this interval, 12 showed an over-all increase and 3 showed a decrease in the number of circulating microfilariae.

Among the treated patients, some of whom had been free of microfilariae for 12 months, none presented untoward symptoms referable to treatment.

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HYPERENDEMICITY OF SCHISTOSOMIASIS JAPONICA ON LEYTE ISLAND, P. I.

F. Bang,[1] M. S. Ferguson,[2] N. G. Hairston,[3] and O. H. Graham[4]

The presence of human infections by the blood fluke *Schistosoma japonicum* on Leyte Island was first reported by Garrison in 1907 (1). From a survey of Leyte and other islands reported in 1941, Tubangui and Pasco (2) estimate that 20% of the people living in an endemic area are infected with schistosomiasis.

Our surveys of five barrios located on the eastern side of Leyte during April and May 1945 revealed that over 80% of the children 10 years of age or older can be proven positive on the basis of only one stool examination if the sedimentation technique is used. Since repeated stool examinations would increase the per cent found positive and since chronic cases of schistosomiasis often produce negative stools for long periods of time, it is our belief that everyone living in the endemic areas of Leyte becomes infected before reaching the age of 15. This conclusion is supported by the habits of the people. They bathe, wash and wade in infected streams almost every day.

Table 1 is a summary of the data obtained from the barrios of Guinarona and Tabontabon (Muncipality of Dagami), the barrio of Limbujan (Muncipality of Tanauan) the barrio of Union (Muncipality of Dulag) and the barrio of Tarragona (Muncipality of Abuyog). One stool examination was made on each individual. If no ova were found in a direct smear a portion of the faecal sample was sedimented and one examination made. The presence of mature ova was the criterion for a positive diagnosis.

Adults as a group had a lower percentage of infection than the children. This difference is statistically significant and is not explained by any predominence of adults examined in one barrio.

TABLE 1

Age and sex distribution of S. japonicum infections

Age	Males		Females		Combined	
	No. studied	% pos.	No. studied	% pos.	No. studied	% pos.
0–2	4	25	6	0	10	10
3–5	4	25	6	50	10	40
6–10	58	77	58	79	116	78
11–15	42	88	59	80	101	84
16 plus	38	71	51	64	89	67

It may reflect the immunity known to develop in other parasitic diseases.

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TRANSFUSIONS OF RED CELLS IN MALARIA

An Experimental Study in Ducks[1]

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The anemias both acute and chronic associated with malarial infections in man frequently are significant. The acute anemia accompanying *P. knowlesi* infection in monkeys is considered to be the basis for the development of the pathological changes (1). The acute lesions in the duck infected with *P. lophurae* also are considered to be the result of the acute and the severe anemias (2). The pathological lesions observed at autopsy in a child infected with *P. falciparum* are similar to those found in fatal cases of shock (3). The shock is considered to be secondary to the acute anemia resulting from the rapid destruction of the red cells by the plasmodia (3). The lesions at autopsy are similar in monkeys infected with *P. knowlesi*, ducks infected with *P. lophurae* and a child infected with *P. falciparum*.

A decrease in the number of red cells, accompanied by a diminution in hemoglobin, is a characteristic feature of all types of acute malarial infections according to Hewitt (4). This fact was demonstrated in birds by Ben-Harel (5), Young (6), and Terzian (7). Hill concluded from her studies on pigeons infected with *P. relictum* that death resulted from the anemia (8).

The significance of shock in P. falciparum infection is indicated by the studies of Rigdon (1–3) and Kean and Smith (9). The presence of shock in cases of malaria suggest the possibility that transfusions may be of value in the treatment of certain cases of malarial infection. Kean and Smith (9) emphasized the results they obtained by treating certain of their patients for shock along with the use of specific therapy for the plasmodium. Red cells appear to be indicated in the treatment of the shock in such cases in preference to plasma. Although it may be that factors other than those resulting from the acute anemia play a rôle in the production of the clinical manifestations in acute cases of malaria it appears at the present time that red cells would be preferable to combat shock in these cases of acute malaria.

A group of ducks infected with *P. lophurae* were used to observe the effect of transfusions of red cells upon the course of their infection. The results of this study are reported in this paper.

METHODS AND MATERIALS

P. lophurae was the strain of malaria used to infect 20 white Pekin ducks. These birds varied in age from two to twelve weeks. The blood used for the inoculum was obtained from the heart of highly parasitized birds. It was diluted with an equal amount of a 2.0% solution of sodium citrate in physiological saline. One half cubic centimeter of this citrated blood was given intravenously into a leg vein. There were approximately 300 parasitized cells per 500 red blood cells in the donor birds at the time of bleeding. The parasitemia was followed by counting the number of parasitized cells in 500 red cells. Blood for these counts was obtained from the legs and web of the feet. The smears were stained with a combination of Giemsa's and Wright's stains. The total red cell counts were made by standard methods. Hayen's fluid was used for the diluent. Reticular counts were made on the same smears used for the parasite counts. A young cell was considered to have a round and larger nucleus than an adult cell. The cytoplasm of these young cells stained a reddish-blue when compared with the normal adult erythrocyte.

Blood used for transfusions was obtained from the heart of normal adult ducks. This blood was diluted with an equal amount of a 2.0 per cent solution of sodium citrate in physiological saline. The blood was kept in the ice box during the time that it was used. In the latter transfusion experiments a greater number of red cells was obtained by centrifuging the citrated blood and removing a part of the plasma. Seventy cubic centimeters of the plasma were added to thirty cubic centimeters of packed red cells.

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TABLE 1

Data on transfused and non-transfused ducks

BIRD NUMBER	NUMBER OF PARASITIZED CELLS AT PEAK PER 500 R B C	TIME PEAK REACHED	TIME OF DEATH	DROP IN NUMBER OF PARASITES FOLLOWING PEAK	INTERVAL BETWEEN LAST COUNT AND DEATH	TRANSFUSION	REMARKS
		days	*days*				
172	460	9	Killed 13th	+		+	Count was 425 on 7th day—240 on 8th and 460 on 9th. Blood diluted with citrate.
174	370	6	7	+	1½ hrs.	+	Blood was diluted with citrate.
176	415	6	6	+	immediately	+	Blood was diluted with citrate.
179	375	5		+	1 hr.	+	Killed by giving too much blood on the 7th injection. Blood was diluted with citrate.
181	410	5		+	1 hr.	+	Died after 2 cc. blood injected at the time of first transfusion.
184	415	6	7	+	3 hrs.	+	Blood was concentrated.
193	460	6	Killed 21st	+		+	Blood was concentrated.
487	430	6	9	+	20 hrs	+	Blood was concentrated.
488	425	6	7	+	10 hrs.	+	Blood was concentrated.
489	445	6	8	+	14 hrs.	+	Blood was concentrated.
608	375	6	Killed 31st	+		+	Blood was diluted with citrate.
495	265	7	7	0	3 hrs	0	This bird died with a lower parasitemia and more red cells than any other bird.
496	375	7	8	0	12 hrs.	0	
497	425	7	8	0	12 hrs.	0	
580	380	5	Discarded 13th day	+		0	
582	355	5	6	+	4 hrs.	0	
586	490	6	7	+	6 hrs	0	
587	490	7	7	0	6 min.	0	
588	495	6	6	+	20 min.	0	
589	405	6	7	+	6 hrs.	0	

The amount of blood used at each transfusion, the number of transfusions and the time at which they were given varied with the different ducks. A duck weighing 750 grams was given three transfusions of 10.0 cubic centimeters each within a period of 21 hours. The blood was injected slowly.

EXPERIMENTAL

The course of the parasitemia and the red cell counts were followed in nine ducks. The graph as shown in figure 1 illustrates the typical type of parasitemia and the anemia observed in these ducks that succumb to the infection. The number of parasites present at the peak of the infection, the time at which the peak is reached and the time of death of all the ducks are shown in table 1. There is a severe and a rapidly developing anemia in all infected birds. In a majority of the ducks the number of parasites decrease preceding the time of death. This diminution in the number of parasites follows the drop in the erythrocyte count. The interval varies from 2 to 24 hours. Only one bird, number 495, died with a low parasite count and a relatively high red cell count. The parasite peak for all the birds is between 355 and 490 per 500 red cells. 500,000 erythrocytes is considered to be the approximate number at the time most of the birds die. There is not an absolute parallelism between the number of parasitized

TABLE 2

EXPERIMENTAL DAY	TIME	RED BLOOD CELLS	PARASITIZED CELLS PER 500 RED BLOOD CELLS
2	A.M.	2,300,000	11
3	A.M.	2,290,000	38
4	9:30 A.M.	2,220,000	87
	3:30 P.M.	2,190,000	150
5	9:30 A.M.	1,830,000	235
	3:30 P.M.	1,800,000	360
6	9:30 A.M.	1,360,000	450
	4:00 P.M.	990,000	370
	9:15 P.M.	580,000	375
	11:15 P.M.		360
7	1:15 A.M.		375
	3:30 A.M.		350
	6:15 A.M.		300
	9:30 A.M.	430,000	230
	4:40 P.M.	Dead	

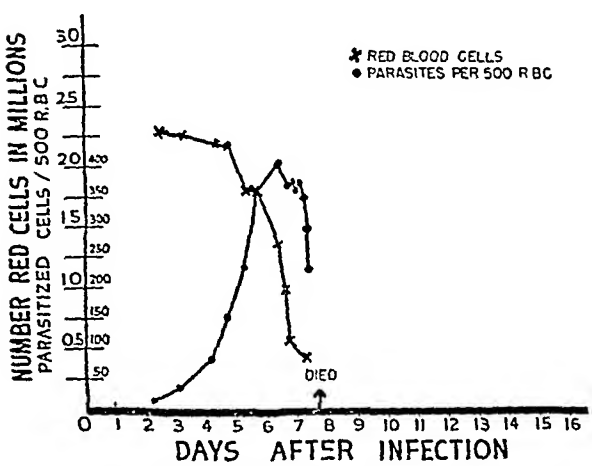

FIG. 1. Duck 589—A typical curve showing the decrease in the number of red cells and the rapid increase in the degree of parasitemia. The number of parasitized cells decrease following the peak of the infection.

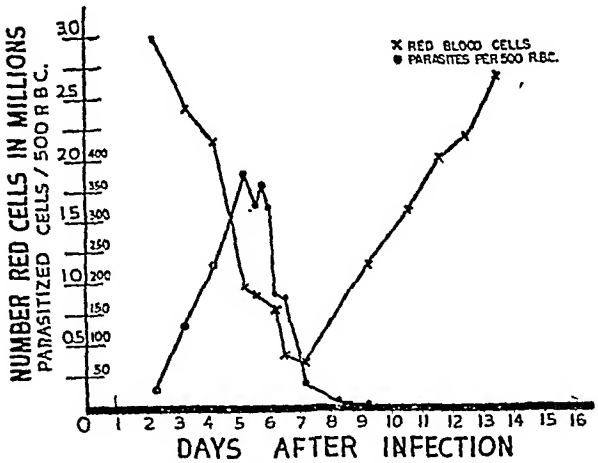

FIG. 2. Duck 580—There is a rapid replacement of red blood cells in the ducks that survive a severe malarial infection. The relation to the parasitemic curve is similar in the ducks that survive to those that die as illustrated in figure 1.

TABLE 3

EXPERIMENTAL DAY	TIME	RED BLOOD CELLS	PARASITIZED CELLS IN 500 RED BLOOD CELLS
2	10:15 A.M.	3,070,000	24
3	10:15 A.M.	2,420,000	130
4	10:15 A.M.	2,140,000	230
5	10:15 A.M.	960,000	380
	2:15 P.M.	890,000	330
	9:30 P.M.		360
	11:00 P.M.		325
6	10:15 A.M.	790,000	185
	3:00 P.M.	400,000	175
7	9:15 A.M.	350,000	37
8	9:30 P.M.		5
9	A.M.	1,140,000	few
	P.M.		0
10		1,580,000	0
11		2,010,000	0
12		2,170,000	0
13		2,660,000	0

cells, the severity of the anemia, and the time of death; however in the ducks in which the complete data are obtained these factors are very closely related. The protocol (table 2) on duck 599 illustrates this.

TABLE 4

EXPERIMENTAL DAY	TIME	R.B.C.	PARASITIZED CELLS IN 500 R.B.C.'S	YOUNG RED CELLS PER 500 CELLS	TRANSFUSION	
					Time	Blood
						cc
3	9:00 A.M.	2,620,000	47			
4	9:00 A.M.	2,410,000	160	28		
5	9:00 A.M.	2,210,000	300	47	12:00 noon	5.0
	12:00		250	67	8:00 P.M.	5.0
	8:00 P.M.		305	90	1:00 P.M.	5.0
6	12:30 A.M.		335			
	4:00 A.M.		315	88	4:00 A.M.	5.0
	9:00 A.M.		355		9:30 A.M.	5.0
	1:30 P.M.		460	103	1:30 P.M.	10.0
	5:00 P.M.		390		5:00 P.M.	10.0
	9:30 P.M.		350	112	9:30 P.M.	10.0
7	2:00 A.M.		335	94	2:00 A.M.	10.0
	7:00 A.M.		310		*	
	9:00 A.M.	2,410,000	315	73		
	11:00 A.M.	2,350,000	315			
	3:30 P.M.	1,750,000	300			
	6:00 P.M.		240	63		
	8:00 P.M.		260			
	10:15 P.M.		295	77		
8	3:30 A.M.		315			
	7:00 A.M.		285	80		
	9:00 A.M.	1,840,000	270	71		
	1:00 P.M.		310	81		
	3:00 P.M.	1,660,000	310			
9	9:00 A.M.	940,000	370	100		
	4:00 P.M.	570,000	315	188		
10	9:00 A.M.	740,000	230	310		
	3:00 P.M.	660,000	111	430		
11	9:00 A.M.	610,000	94	390		
	4:00 P.M.	1,010,000	51	452		
12	9:00 A.M.	1,510,000	31	412		
14	9:00 A.M.	1,890,000	10	203		
	4:00 P.M.	1,950,000	8	206		
15	9:00 A.M.	2,540,000	0	205		
21	Killed					

* Weight 827 grams.

One duck, #580, survived from the group of nine birds (table 3). The red blood cell counts and the course of the parasitemia are shown in figure 2. The course of the infection in this bird is identical with that of bird 589 as shown in figure 1, except the number of red cells began to increase on the seventh day following a decrease to 350,000 cells. The number of parasitized cells rapidly decreased and none was demonstrated in the peripheral blood four days after the peak of the infection. The number of erythrocytes rapidly increased and seven days after the time of the greatest anemia the count had returned within the range of normal.

The type of curve following the peak of infection in ducks that either die or spontaneously recover from *P. lophurae* infection is significant when the course of the infection is followed in birds given transfusions. The parasitemia rapidly and pro-

gressively decreases following the peak in all the ducks used in this experiment.

EFFECT OF TRANSFUSIONS UPON THE COURSE OF THE PARASITEMIA IN P. LOPHURAE INFECTION IN THE DUCK

Eleven ducks with malaria were transfused. Duck 181 died after 2.0 cc. of blood was injected. Duck 179 died during the time of injection. This bird was given 30 cc. during the preceding 12 hours and died after receiving approximately 14 cc. at a single injection. Three, numbers 172, 193, and 608, of the remaining 9 ducks given transfusions

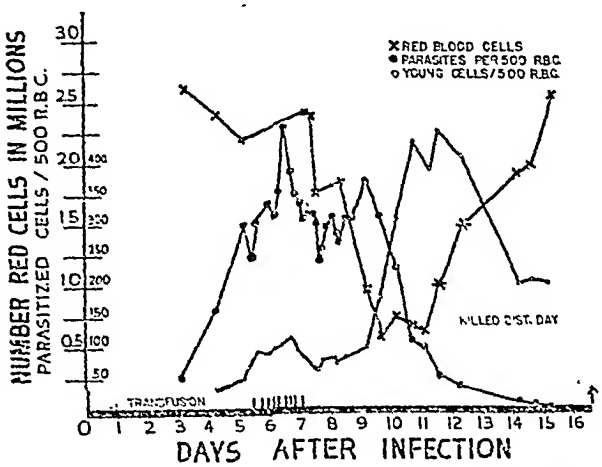

FIG. 3. Duck 193. The development of both the anemia and the parasitemia is influenced by the intravenous injection of large amounts of normal blood. The number of parasites decrease following the peak of parasitemia, however, the rate is greatly modified. The time of the occurrence of the decrease in the number of red cells follows the original decrease in the number of parasites. In the normal bird the red cells decrease preceding the time of the fall in the parasite count. The relation of the parasitemia to the anemia in the terminal phase of the infection is similar to that in any bird that survives.

survived the infection (table 1). All the birds appeared greatly improved almost immediately following a transfusion. The greatest improvement occurred in the most anemic birds. There is no question of immediate improvement in the appearance of the birds following the transfusion. This is indicated in the protocol on duck 193 (table 4).

The protocol on bird 193 shows the quantity of blood necessary to keep the red cell count constant. The duck weighed 827 grams and was given 65 cc. of blood containing 30 cc. of packed cells in 70 cc. of plasma during an interval of 48 hours. Duck 580 showed a decrease of 1,180,000 red blood cells during a period of 24 hours when the parasitemia was 230 to 380 per 500 red blood cells (fig. 2).

The effect of transfusions on the course of the parasitemia in duck 193, is shown in figure 3. This shows a typical curve for the normal increase in the parasitemia. On the sixth day the peak was

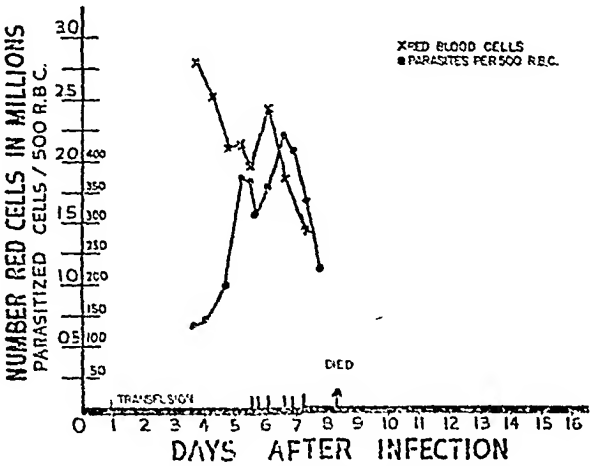

FIG. 4. Duck 489. The number of red cells and parasites increase following the transfusion. Apparently an insufficient amount of blood was given to this duck. The relation of the fall in red cells to the drop in the number of parasitized cells is the same as that in a non-transfused bird.

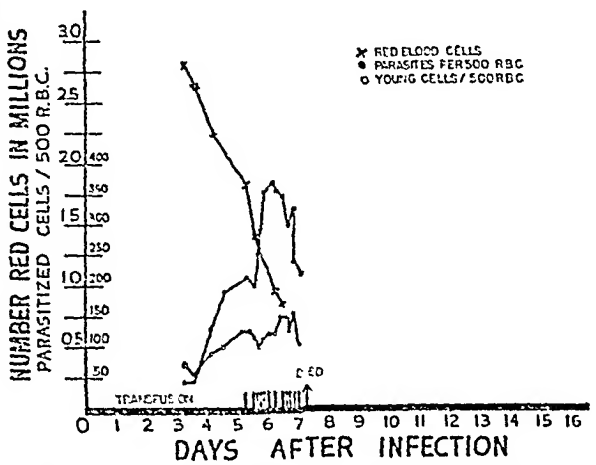

FIG. 5. Duck 174. The amount of blood given to this duck did not influence either the course of the anemia or the parasitemia. To influence the course of the parasitemia a sufficient quantity of blood must be given to inhibit the development of the anemia.

reached and the number of parasites rapidly decreased during the following 24 hours to a level of 240 parasitized cells. At this time the number of parasitized cells slowly increased over a period of 24 hours to reach 370. The number of parasitized cells rapidly decreased for five days at which time none were present in the circulating

blood. The second rise in the parasite count followed the transfusions. The final drop in the number of parasitized cells occurred 24 hours following the decrease in the number of red cells. This second drop in the number of parasitized cells is identical with the drop that occurs in birds that are not transfused as shown by duck 580 in figure 2. Duck 489 (fig. 4) shows a second peak following transfusion which is greater than the first.

Some of the birds given transfusions failed to show any variation in the course of the anemia and the parasitemia as shown by duck 174 in figure 5. The amount of blood injected apparently was insufficient to produce an increase in the total red count in the presence of the severe malarial infection. Death occurred following the typical decrease in the number of erythrocytes and in the number of parasitized cells in the ducks given small amounts of blood the same as it does in untreated birds.

The young cells rapidly increase in number following the anemia in the ducks that survive the infection. It is interesting to observe the rapidity in which these young cells become mature (fig. 3).

DISCUSSION

The number of ducks transfused and the number of survivals in this experiment are too few to be of statistical significance. The results do show, however, that the course of *P. lophurae* infection in ducks may be changed by the injection of duck blood. Furthermore these studies indicate that a large number of red cells are destroyed within a short interval by *P. lophurae* when the parasitemia is high.

It is also shown in these results that the number of parasites may be kept at a high level provided a sufficient quantity of normal red blood cells are injected to keep the total erythrocyte count within the range of normal. Duck 193 illustrates this fact. The decrease in the number of parasitized cells following the peak of the infection has been considered by many investigators to be a manifestation of immunity. Ben-Harel (5) in discussing this problem of phagocytosis says, "It would therefore seem probable that the great reduction in the number of parasites is due to their actual destruction by fixed tissue cells, as well as by the circulating phagocytes. . . . Phagocytosis is probably the most important factor in causing the disappearance of the parasites from the peripheral circulation." Apparently there is no reason why phagocytosis could not progress normally in ducks given transfusions and thereby deplete the blood stream of parasitized red cells. Since this does not occur it is suggested by this study that factors other than phagocytosis may be significant in producing the decrease in the parasitemia following the peak of the infection.

The pathological lesions occurring in ducks infected with *P. lophurae* are similar to those occurring in shock (2). It has been suggested that this shock results from the destruction of the red cells by the parasites. It may be that this anoxia is detrimental to the parasite as well as to the host. Marvin and Rigdon (10) have shown recently that the quantity of glucose decreases preceding death in ducks with a severe malarial infection. This diminution in glucose likewise may contribute to the failure of multiplication of the parasites following the peak of the infection. The studies of Russell and Mohan (11) indicate that certain immune substances develop in birds infected with certain of the plasmodia. The mechanism of their effect in malarial infection, however, is still open to question.

The rapid regeneration of the erythrocytes as observed in this experiment is similar to that observation by Terzian in *P. lophurae* infections in chicks (7). The relation of the decrease in parasites to the anemia in this experiment is similar to that made by other investigators (4, 7). The results of this study and the pathological studies reported elsewhere (1–3) indicate that there is a definite relationship between the anemia in malarial infections and death.

Transfusions of red blood cells cannot be considered as a specific therapic procedure in malaria. These red cells may aid in the transportation of oxygen to the tissues during a crucial period in which a specific therapeutic agent is acting upon the plasmodium and thereby death may be prevented from anoxia. It would be useless in a case of malaria to kill the plasmodium and have the patient to die from anoxia.

SUMMARY

The course of the parasitemia in ducks infected with *P. lophurae* is changed by the injection of duck blood. The parasitemia may be kept at a high level by the injection of large amounts of duck blood. Transfusions apparently have little if any effect upon the ultimate outcome of *P. lophurae* infection in ducks.

The relationship of transfusions to the course of parasitemia and to the mechanism of immunity in *P. lophurae* infection in ducks is discussed.

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THE Rh FACTOR IN BLACKWATER FEVER

A Preliminary Note[1]

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The idea that some form of sensitization might be associated with the development of blackwater fever has been previously suggested by Foy, Altmann, Barnes and Kondi (1), and Fernán-Núñez (2). The idea expressed in this preliminary note was developed from the studies of these workers and those of Whitmore (3, 4).

In 1940, Landsteiner and Wiener (5) described the existence of a new factor in human bloods (the so-called "Rh Factor"). In 1941, Burnham (6), and Levine, Katzin and Burnham (7) associated the Rh factor with erythroblastosis fetalis.

With these basic observations as a nucleus, let us now consider the theoretical possibilities of the Rh factor, or an Rh-like substance, in the causation of blackwater fever.

Following the demonstration of the Rh factor in the blood, Landsteiner and Wiener (8) showed that the factor (agglutinogen) was inherited as a simple Mendelian dominant. Later, Levine, Vogel, Katzin and Burnham (9) demonstrated the isoimmunization pathogenesis of erythroblastosis fetalis. Previous to that time, Wiener and Peters (10) had demonstrated that Rh negative individuals who had been transfused with the blood of Rh positive donors develop antibodies (anti-Rh agglutinins) to the Rh factor which, upon repeated transfusions of Rh positive bloods, were capable of producing severe reactions. These reactions are generally of the hemolytic type, clinically manifested as a hemoglobinuria.

Schittenhelm, Erhardt and Warnat (11) have demonstrated in dogs and rabbits that the serum potassium rises to a very high level during acute anaphylactic shock. Pinelli (12) has reported a marked increase in serum potassium during the fever period in tertian and malignant tertian (aestivo-autumnal) malaria. It is becoming increasingly well recognized that the paroxysm in human malaria in some respects resemble those of anaphylactic shock. Recently* Kean and Taylor reported six cases of algid malaria presenting the typical syndrome of medical (surgical) shock. The authors did not attempt to explain the reaction other than to say that it was probably a complicated combination of factors.

That plasmodia are antigenic is borne out by the fact that a certain degree of immunity may be acquired in malaria, probably due to stimulation of the macrophage system by the parasites. The antigenicity of the malaria parasites is further demonstrated by the work of Eaton and Coggeshall (13) who have developed a complement fixation test for the diagnosis of malarial infections, using *Plasmodium knowlesi* as antigen. This reaction is group-specific rather than species-specific, i.e., sera from patients infected with *P. vivax* or *P. falciparum* react in the same way with the antigen as the homologous sera. However, the specific nature of the antibody in malaria has been demonstrated by Dulaney and Stratman-Thomas (14) These workers, by means of absorption tests showed that absorption with Wassermann antigen removes the so-called syphilitic reagin without affecting the reactivity of the serum with the malaria antigen to a significant degree.

Yorke and Nauss (15) have demonstrated that the suppression of urine in blackwater fever is considerably facilitated by any factor which tends to lower the blood pressure. It is a well known fact that hemolytic shock following the transfusion of incompatible bloods often results in hemolysis of the erythrocytes, followed by a sharp fall in the blood pressure and derangement of renal function.

By putting all of these pertinent facts together, it appeared that it might be probable that the malaria parasites might possess a strong Rh-like substance which would be capable of sensitizing a certain percentage of Rh negative individuals in

[1] Read at the 38th Annual Meeting of the Southern Medical Association, November 13–16, 1944.

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* 38th Annual Meeting of the Southern Medical Association, November 13–16, 1944.

much the same manner as sensitization by transfusion of Rh positive blood, or by an Rh positive fetus. The Rh negative individual once sensitized by the parasites, i.e., anti-Rh agglutinins once produced, subsequent relapses of the infection (each time bringing the Rh-like plasmodia into the circulation) would ultimately bring about a hemolytic reaction (hemoglobinuria), clinically manifested as blackwater fever.

RACIAL INCIDENCE OF BLACKWATER FEVER AND THE Rh FACTOR DISTRIBUTION

First let us consider the racial distribution of the Rh factor and incidence of blackwater fever among various races.

TABLE 1

(Taken from Levine (16))

RACE	PERCENTAGE	
	Rh +	Rh −
Whites	85.0	15.0
Negroes	95.5	4.5
Chinese	99.3	0.7

TABLE 2

EACH 1000 CASES OF MALARIA (RACE)	NUMBER OF Rh NEGATIVE INDIVIDUALS EXPECTED PER 1000 CASES OF MALARIA (see table 1)	THEORETICAL NUMBER OF INDIVIDUALS SENSITIZED, I.E., NUMBER OF CASES OF BLACKWATER FEVER EXPECTED PER 1000 CASES OF MALARIA (2–4 PER CENT OF THE Rh NEGATIVE INDIVIDUALS)
Whites	150	3–6
Negroes	45	0.9–1.8
Chinese	7	0.14–0.28

A study made by Levine (16), and others, shows a great variation in Rh factor according to race (table 1).

According to Wiener (17), the frequency with which isoimmunization occurs is only in about 2 to 4 per cent of the Rh negative individuals, except under the unusual circumstances of 20 or more transfusions. In this case, the incidence of sensitization could be much higher.

Using the figures of Levine (table 1) and those of Wiener (17), let us calculate the number of cases of blackwater fever which we might expect among each 1000 cases of malaria (malignant tertian) among various races (table 2).

Let us review some statistics on blackwater fever and then compare them with the above calculated (theoretical) figures, based on the theory of Rh sensitization.

According to Craig (18), Manson-Bahr (19) and others, blackwater fever is far more common among whites than among blacks in West Africa. Giglioli (20) states: "In Rhodesia and other African regions where the disease (blackwater fever) is common, the native negroes are practically immune." Simmons, Whayne, Anderson and Horack (21) state that blackwater fever is frequent among Europeans in New Guinea but rare among the natives, which are made up of Papuans and Negritas (both of which are negroid tribes). According to these same authors, blackwater fever

TABLE 3

YEAR	NATIONALITY OR RACE	NUMBER OF CASES OF HAEMOGLOBINURIC FEVER PER 1000 PER YEAR
1905	Americans	3.30
1906		1.90
1907		0.70
1908		0.39
1909		0.48
1905	Europeans	5.00
1906		5.50
1907		1.25
1908		5.88
1909		11.36
1905	Negroes	0.33
1906		0.59
1907		0.28
1908		0.00
1909		0.25

occurs only among the white population of the New Hebrides, the native population here consisting almost entirely of native Papuans; while in China proper blackwater fever is rare. Supporting the latter statement, Seaton (22) states: "Blackwater fever only occur sin Formosa, Yunnan and Hainan Island, with an occasional case reported from the South China ports." Statistics of Deeks and James (23) from the Canal Zone (table 3) are of great interest.

The estimated figures in table 2 for the theoretical number of cases of blackwater fever, based on Rh factor are likewise of interest when compared with those presented by Potter (24) on the racial mortality of erythroblastosis fetalis. Potter's results are tabulated in table 4.

Erythroblastosis fetalis is known to be very rare among the Chinese. (See Levine and Wong (25).)

Table 5 will summarize the data relative to the racial distribution of blackwater fever (calculated and actual) and erythroblastosis fetalis.

MULTIPLE CASES OF BLACKWATER FEVER IN THE SAME FAMILY

Landsteiner and Wiener (26) have demonstrated that if both the mother and the father are Rh negative, all offspring from this mating will be Rh negative. This observation may explain the occurrence of blackwater fever among members of the same family, as reported by Whitmore (3), and Giglioli (20). Manson-Bahr (19) states: "The occurrence of several cases of blackwater fever in the same family may not be pure coincidence, but probably is explained by exposure to a common factor."

TABLE 4

RACE	DEATHS FROM ERYTHROBLASTOSIS
Whites	37
Negroes	2

TABLE 5

	RATIOS white:negroes
Cases of blackwater fever (calculated) (table 2)	30–60: 9–18
Cases of blackwater fever (actual) (table 3)*	33–59:2.5–5.9
Deaths from erythroblastosis (actual) (table 4)	37 :2

* The exceptionally high and low figures shown in table 3 have been excluded from these ratios.

It is of interest to note in connection with the familial occurrence of blackwater fever that, according to the studies of Whitmore and Giglioli, no household conditions could be observed which would explain why members of certain families should have blackwater fever any more than other families throughout the same district, and who were living under the same environmental conditions. Also, that certain members of the same family may suffer repeated attacks of blackwater fever while other members of the same family may remain free from a single attack. I believe that these observations may be explained on the basis of the hypothesis set forth in this preliminary communication.

STILLBIRTHS AND BLACKWATER FEVER

Several instances where stillbirths and neonatal deaths were preceeded by attacks of blackwater fever have been cited in the literature. Thomas and Millen (27) and Foy and Kondi (28) have reported such instances. It is well known that stillbirths and neonatal deaths are not uncommon when an Rh negative mother has previously been transfused with Rh positive blood, or has been sensitized by the birth of a previous Rh positive fetus. Therefore it appears logical to assume that if the malaria parasites possess an Rh-like substance, that an Rh negative mother previously sensitized by repeated paroxysms of malaria, to the extent that hemoglobinuria had developed, might suffer the same reactions as if sensitized by previous transfusions of Rh positive blood.

THE RÔLE OF ANTIMALARIAL DRUGS IN BLACKWATER FEVER

The rôle of quinine therapy, and to a lesser degree atebrine therapy, in blackwater fever has long been a subject of debate. There is little doubt but that these drugs are responsible for precipitating the majority of attacks of blackwater fever, however, numerous cases are recorded in the literature where these drugs had not been administered prior to an attack of blackwater fever. Whitmore (3), So (29), Fernán-Núñez (30), and numerous others have reported such cases. Therefore it is apparent that another factor, other than drug therapy, is active in initiating the hemolytic reaction of blackwater fever. In concluding this preliminary note, it might be stressed that the explanation herewith presented is in all probability not as simple as it might appear. Additional theoretical and experimental verification of the idea presented in this preliminary note are already under way. A subsequent report will be made as soon as experimental observations warrant their release.

Since submitting this preliminary note for publication it has been possible, due to the combined cooperation of the United States Navy, the United States Army, and the United Fruit Company, for John Elliott, Captain, Sanitary Corps, A.U.S. and myself to make some very brief preliminary ob-

servations in Banes, Cuba. These studies were very encouraging.

Due to the very limited time available, the only members of Cuban families previously reported by Whitmore (3) which we were able to contact consisted of a brother and sister of the Tamayo family. Both of these had previously had blackwater fever, and both were Rh negative.

It is intended to extend this study on the familial occurrence of blackwater fever and Rh factor, and also to undertake several other lines of investigation which suggest themselves.

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SIMULTANEOUS VACCINATION AGAINST BACILLARY DYSENTERY AND CHOLERA WITH TOXOID-VACCINE[1]

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This investigation was inaugurated by the Health Service of the Czechoslovak Army eight years ago, for the purpose of producing a reliable combined vaccine against cholera and dysentery, which might be used in the eventuality that troops would be stationed in areas where these diseases are prevalent. Due to events preceding the present holocaust the experiments were necessarily interrupted and reopened during recent years in the United States.

A vaccine was sought which would protect against infections with *V. cholerae*, *Sh. dysenteriae* and *Sh. paradysenteriae* Y, the latter being chosen because of its ability to confer partial immunity against the most common Shigella strains, namely *Sh. paradysenteriae* V, W and Z.

In order to determine which type of *Sh. dysenteriae* vaccine would confer maximum immunity, alcohol and heat killed organisms, and formolized and phenolized bacillary suspensions were tested. Organisms extracted with trichloracetic acid, formamate and diethylene glycol were utilized. Lastly, culture filtrates treated with formol, phenol and potassium permanganate, and their combinations with the aforementioned suspensions and extracts were examined. Mouse protection tests showed that the strongest degree of immunity was afforded by the use of a combination of alcohol killed organisms and formolized culture filtrate.

The same procedures were followed using *Sh. paradysenteriae* Y, with the exception of experiments involving the use of culture filtrates. The most effective vaccine was that prepared from alcohol treated organisms.

Immunizing agents against cholera were prepared with Inaba, Ogawa and El Tor strains. Parallel experiments were set up using heat, alcohol, formol and potassium permanganate killed organisms. Gallut's and Strong's lysates; Besredka's vaccine; culture filtrates treated with formol, alcohol and phenol and their combinations were tested on rabbits, mice and guinea pigs. The best results were achieved by the use of formol or phenol killed Inaba organisms combined with formolized filtrates of Inaba or El Tor strains. Because filtrates of *V. El Tor* were less toxic, and stimulated the production of antibodies against the hemodigestive and necrotoxic action of *V. cholerae* to a higher level, this filtrate was used in the final vaccine in combination with the formol killed Inaba organisms.

The vaccines were tested to determine the length of time they might be stored without loss of immunizing power. The mixture of formolized organisms with formol treated filtrates proved to be the most stable. All vaccines, however, retained their immunizing ability much longer when desiccated in vacuum.

Three groups of human volunteers, twenty in each group, were injected with three types of immunizing agents: phenolized and heat killed vaccine, formolized filtrate and a mixture of formolized organisms with formolized filtrate. The antibody production was checked by mouse protection tests. Formolized organisms combined with formolized filtrate gave the strongest antibody production and that which lasted for the longest time.

A group of eighteen volunteers was immunized with a mixture of antidysenteric and anticholeric vaccines which had proved the most efficacious. The reactions did not exceed those observed when only one of the vaccines was used Mouse protection tests proved there was a satisfactory increase of antibody formation which persisted for a considerable length of time.

From these preliminary experiments it may be concluded that a combined vaccine against cholera and dysentery can be prepared which stimulates the production of protective antibodies to a high level. These findings indicate that continuation of these experiments is fully warranted.

[1] Read at the 40th Annual meeting of the American Society of Tropical Medicine, St. Louis, Mo., November 13–16, 1944.

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THE PNEUMONIAS IN PANAMA

A Study of Five Hundred Consecutive Cases

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Because of the prevalence of pneumonia[3] in the Canal Zone, the study of a series of cases was undertaken. It was felt that an analysis of an adequate number of cases would be indicative of the types of pneumonia liable to occur in the tropics. Since this study has been made during a limited period and as the possibility of annual variation is recognized, the results are not offered as being representative of pneumonias in all locales of the torrid zone.

Although a thorough review of the literature is not within the scope of this presentation, it is well to mention that there have been numerous studies of the pneumonia problem in tropical zones. Reports have been submitted from many scattered areas of the globe and a variety of causative agents has been uncovered, showing that the problems involved often resemble those of the temperate zone. L. B. Bates (1) described a hemorrhagic bronchopneumonia resembling pneumonic plague due to *B. mucosus* capsulatus in Panama. The epidemics of the pulmonary type of bubonic plague are too well known to require any further comment. The incidence of lobar pneumonia in Malaya was studied by Winchester (2). In the Phillipine Islands a study of pneumonia in children under three years of age was completed by J. Albert (3). Other scientific contributions from South Africa (4, 5), Mexico (6), Netherland India (7), Hawaii (8), Porto Rico (9), Egypt (10), and India (11) attest to the universality of this problem. The report on tropical pneumonia in the Quarterly Bulletin of the League of Nations (12) merits attention.

The effect of climate has been commented upon by numerous investigators. It has been generally accepted that the incidence of pneumonia is greatest in the temperate zone during the winter and early spring seasons. There seems to be some difference of opinion as to its prevalence in tropical regions, but exception is taken to the statement that pneumonia is rare. It is true, however, that studies have been more extensive, facilities more accessible, and reports in the literature more abundant in the temperate zone. A combination of these factors may tend to minimize or even dwarf the problem as it exists in warmer climes. A report (13) by the medical department of the United Fruit Co. at the International Conference on Health Problems in Tropical America is revealing. In the report of deaths per 1000 employees during 1923 the following causes were listed: Penumonia 5.5, tuberculosis 2.2, dysentery 1.9, and malaria 1. As for influenzal pneumonia, the same report indicates that in consequence of international transportation, the disease is confined to no country or hemisphere. Tropical areas are not exempt.

In Panama the first reported large scale epidemic of pneumonia occurred when work started on the Canal. Scott (14) in his History of Tropical Medicine extols the excellent and energetic measures of Gorgas in combating this disease. It was as destructive to the negro as yellow fever was to the white man. By segregating those attacked, putting an end to overcrowding, improving their dwellings and food, he succeeded in eradicating the epidemic forms of the disease. Deeks (15) submitted an analysis of 574 of these cases. The mortality during this era was appalling: 37% for negro West Indians, 58% for Panamanians, 52% for Colombians, and 20% for the white race. There seems to be no doubt that whites are more apt to present a greater resistance and ability to recover. Since this period, pneumonitis has been a problem, and several unpublished studies have been made. There have been several minor outbreaks of the disease, particularly among the black laborers who live in camps or barracks. A recent report from this zone was submitted by Campbell (16) who described atypical pneumonitis associated with malaria. In general, social conditions, racial status, and occupational environment seem to be more important than climate; and it may be com-

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[3] Pneumonia and pneumonitis are used as interchangeable terms.

phatically stated that pneumonia is not only a disease of temperate zones, but merits equal attention under all climatic conditions.

METHOD AND MATERIAL

Five hundred consecutive cases of pneumonia admitted to Gorgas Hospital during an eighteen-month period (1942–1943) were investigated. Only cases with *primary pneumonitis* were selected in contradistinction to the secondary forms, which follow some dominant or grave illness. Cases associated with cardiovascular disease, chronic pulmonary pathology, operative conditions, and moribund states were discarded. Pneumonitis associated with malaria (17) was considered as a separate entity, and special studies have been completed in this latter group. Clinical cases of pneumonia, where x-ray confirmation or sputum studies were lacking, were not acceptable for this study.

Each patient received the Gorgas Hospital workup, consisting of a complete history; a physical examination; laboratory studies which included a complete blood count, hemoglobin determination, blood serology, blood film examination for malaria, urine examination, and stool examination. In addition, roentgen studies of the chest were made and sputum studies by cultural methods were obtained in all cases. Blood cultures were submitted in the more seriously ill and other examinations were performed for purposes of differential diagnosis.

RESULTS

Type of patient, incidence, seasonal occurrence

As the above factors were not affected by etiology, discussion will concern the group as an entity. Patients were chiefly male adults, averaging 28.4 years in age. They were distributed among three racial groups, white, foreign-white and black, with the black race predominating (43%). The majority were civilian employees of the Panama Canal and approximately 20% were in the military service of the United States.

The incidence of pneumonitis in relationship to the total population of the Canal Zone cannot be calculated at present. However approximately 3% of all admissions (33,913) to Gorgas Hospital during a twelve-month period (April, 1942–March, 1943) were listed as primary pneumonias, offering an index of the prevalence of this disease.

In Panama the months from December to April constitute the "dry season" and the remainder of the year is known as the "rainy season." The peak of admissions occurred during the rainy season and was maintained from July through November.

Etiological agents (Based on Sputum Studies)

Bacteriological studies of sputum by means of cultural methods and typing pneumococci were performed by the Board of Health Laboratory of the Canal Zone. Negative reports included those specimens revealing small numbers of bacteria (mixed flora) commonly found in the respiratory tract of normal individuals. Whenever an organism was abundant and dominant, or pathogenic, it was reported. Sputum specimens were submitted on several occasions in each case.

TABLE 1

Analysis of sputum study

ORGANISM	NUMBER OF CASES	LEUKOCYTOSIS		LOBAR DISTRIBUTION		GOOD RESPONSE TO CHEMOTHERAPY	
		Cases	%	Cases	%	Cases	%
1. Negative	359	117	32	48	13	124	34
2. Pneumococcus							
a. Untyped	30	22	73	7	23	23	76
b. Typed							
I	28						
II	3						
III	4						
IV	2						
V	5 72	58	80	22	30	59	81
VI	5						
VII	9						
VIII	6						
IX	3						
XIV	2						
XIX	3						
XX	2						
3. Streptococcus hemolyticus	31	19	61	3	9	16	51
4. Streptococcus anhemolyticus	8	6	75	1	12	7	87

Of the 500 patients, negative results were obtained in 359 (71+%). Specific organisms were

identified in the remaining 141 cases (28+%) in the following order: Pneumococcus, specific type, 72; *Streptococcus hemolyticus* 31; pneumococcus, untyped 30; *Streptococcus anhemolyticus*, 8. This study was cross-analyzed with leukocytosis, pulmonary distribution, response to chemotherapy in the form of sulfonamides to determine any possible relationship (see table 1). In general leukocytosis, lobar distribution and an adequate chemotherapeutic response were more common in the bacteria group than in the negative culture series. The one exception was detected in the streptococcal groups, where lobar distribution was lowest.

Clinical aspects of negative culture group (359 cases)

The onset was variable, gradual in 64% and acute in 36%. Upper respiratory infections (45%) often preceded evidence of a pulmonary infection by one or more weeks. Such systemic manifestations as feverishness, chills, headache, gastrointestinal symptoms, malaise and general aches occurred in the great majority (90+%). Respiratory symptoms as cough, expectoration and chest pain were prevalent in approximately 70% and hemoptysis was noted in 7% of this group.

The detection of rales by auscultation was the outstanding finding. They were usually fine to medium, moderate in number, inspiratory, and constant, persisting as a rule after cough. The abnormal quality of the breath sound (bronchovesicular to bronchial) and dullness were also important signs. At times the first and only finding was a diminution in the intensity of the breath sound and, therefore, it is considered an important observation. Wheezes, indicative of an associated diffuse bronchitis, and pleural friction rub, pathognomonic of an associated fibrinous pleuritis, occurred in a small percentage of cases. Dyspnea and cyanosis, the marks of severe and toxic cases were relatively infrequent, indicating the benignity of the process as seen in this group. It was interesting to note that 28% of the cases presented no abnormal physical findings and the diagnosis was established by roentgen studies. The impotency of the stethoscope in one-fourth of these cases is therefore recognized.

All patients underwent roentgen studies and lesions were chiefly of lobular distribution in the lower lobes. Although associated regional hilar involvement was common (23%), it was by no means observed as frequently as in other studies. Upper lobe lesions occurred in 27% and presented the problem of differentiation from pulmonary tuberculosis, particularly when resolution was prolonged. Negative tuberculin tests were helpful in ruling out Koch infection in this series. Table 2 represents a summary of the roentgen findings.

Other laboratory findings were of no clinical significance. Facilities for virus studies were not available. All blood cultures (72), submitted in the more seriously ill, yielded negative results. A study of the total white cell count and differential was made during the peak of the illness. Most of the cases (63%) revealed normal responses; leukocytosis with an increase in polymorphonuclear elements was detected in 32% and leukopenia was relatively uncommon (5%).

TABLE 2

Analysis of x-ray findings (negative culture group)

	NUMBER CASES	PERCENTAGE
1. Type of Lesion		
a. Lobar distribution	48	13+
b. Lobular distribution	311	86+
2. Location of Lesion		
a. Single lobes	313	87
(1) Right lower lobe	104	29
(2) Left lower lobe	99	27+
(3) Right upper lobe	55	15
(4) Right middle lobe	31	8+
(5) Left upper lobe	24	6+
b. Multiple lobes	46	12+
(1) Bilateral	30	8
(2) Unilateral	16	4
(a) Right	10	2+
(b) Left	6	1+
3. Complications		
a. Spread (migratory)	21	6
b. Pleural effusion	14	3+
c. Spontaneous pneumothorax	1	0.2
d. Mediastinal lymphadenopathy	1	0.2

Chemotherapy in the form of sulfadiazine or sulfathiazole was employed in the majority of cases (86%). In about one-third (34%) the response to the drug was very satisfactory. The routine consisted of an initial dose of four grams of the sulfa drug, followed by one gram every 4 hours for 48 hours and finally one gram every 6 hours until the drug was discontinued. No chemotherapy was utilized in the very mild forms. Patients with a leukocytic trend exhibited an especially good response, while those without leuko-

cytosis tended to react less favorably. No disproportion in response between the lobular and lobar lesions could be ascertained. Special treatment was required in the critically ill and the infrequent application of such energetic measures as oxygen therapy, blood transfusion, etc., is another index of the moderate course. The fact that nearly 10% of the cases were treated with anti-malarial drugs suggests the similarity of the constitutional manifestations of pneumonia and malaria.

The great majority of cases consisted of mild and moderate forms of the disease and only a small fraction (4+%) was listed as severe. Although the effect of racial influence seemed minimal, it can be stated that in general the course was more severe in the black patients. The average hospital stay was ten days. Approximately 40% of the cases required no further convalescence and the remaining 60% of the patients were confined to quarters for about one week. Complications occurred in 10% of the cases (see table 2) and were chiefly pleuritis and spread of the lesion. There were no serious complications and there was only one death, which could possibly be assigned to the category of atypical pneumonia.

Report of the one death in the negative culture group: A 26-year-old foreign-white laborer was ill for three days prior to hospital admission with chills, fever and productive cough. A clinical diagnosis of lobar pneumonia of the right lower lobe was made and confirmed by roentgen films. Blood cultures and sputum cultures were negative. He did not respond to chemotherapy and ran a toxic down-hill course for seven days, exhibiting cyanosis and dyspnea. He died in terminal pulmonary edema.

Postmortem examination revealed a deep red uniformly solid right lower lobe, rather moist. In the left lower lobe there were scattered areas of bronchopneumonia. On microscopic examination the alveoli were filled with exudate containing polymorphonuclear cells, monocytes and red cells. No dominant bacteria were present. There were also findings of interstitial pneumonitis and peribronchitis. This was the only death suggestive of a non-bacterial pneumonitis; so-called atypical pneumonia.

Clinical aspects of bacterial group

In 141 (28+%) of the 500 cases, organisms were repeatedly identified by cultural studies of the sputum and typing (see table 1), so that an etiological background was specifically established. The group resembled the types of bacterial pneumonia seen in other areas. The onset was acute in 71% of the cases, physical signs were absent in 14%, lobar distribution was noted in 23+%, leukocytosis was present in 74%, and an adequate response to sulfonamides was elicited in approximately 74% of the cases. The following complications were noted: Empyema, pneumococcal, 3; pleural effusion, sterile, 2; purulent otitis media, 2; suppurative pericarditis, pneumococcal, 1; toxic nephritis, 1; meningitis, pneumococcal, 1; purulent conjunctivitis, 1. There were only 11 cases (8%) listed as severe and the rest ran a relatively benign course. There were three deaths in the series (mortality rate of 2.1%) and all were due to overwhelming pneumococcal infections.

Brief report of three deaths in bacterial group: 1. A 47-year-old male, black Jamaican, laborer, was ill for one day prior to admission. He entered the hospital in extremis, dyspneic and cyanotic. His sputum was positive for type I pneumococcus and he had clinical evidence of lobar pneumonia of the right lower lobe. He did not respond to chemotherapy and died after six hospital days.

Postmortem examination demonstrated a lobar pneumonia due to type I pneumococcus. Postmortem blood cultures were positive for type I pneumococcus.

2. A 58-year-old, black Jamaican, laborer, was ill for four days before entering the hospital. On admission he had clinical evidence of a lobar pneumonia of the left lower lobe and signs of meningitis. A spinal tap was performed and purulent fluid was obtained. Type I pneumococcus was cultured from the fluid. He was admitted in extremis and did not respond to sulfadiazine. Death occurred after three hospital days. No postmortem examination was obtained.

3. A 47-year-old, black male, was admitted to the hospital after two days of symptoms. He was seriously ill, had signs of lobar pneumonia of the left lower lobe and did not respond to chemotherapy. He died within forty-eight hours after a fulminating course.

Postmortem examination showed a lobar pneumonia involving the entire left lung due to type V pneumococcus, complicated by a suppurative pleuritis and pericarditis.

CASE REPORTS

Several illustrative case reports are presented:

Case 1. A 28-year-old foreign-white male, Panamanian, laborer was admitted to Gorgas Hospital on

June 22, 1942. He was ill for six days with a dry cough, slight fever, headache and pain in the left chest. Signs of diminished breath sounds and fine inspiratory rales were heard over the left base. Cultures of sputum specimens were negative. Blood cultures were negative. The total white cell count was 4,900 with a differential of 64% polymorphonuclear cells and 36% lymphocytes. All other tests were negative. X-rays (see fig. 1) revealed a pneumonitis of the left lower lobe on June 23, 1942 and incomplete resolution with residual pleuritis on July 15, 1943.

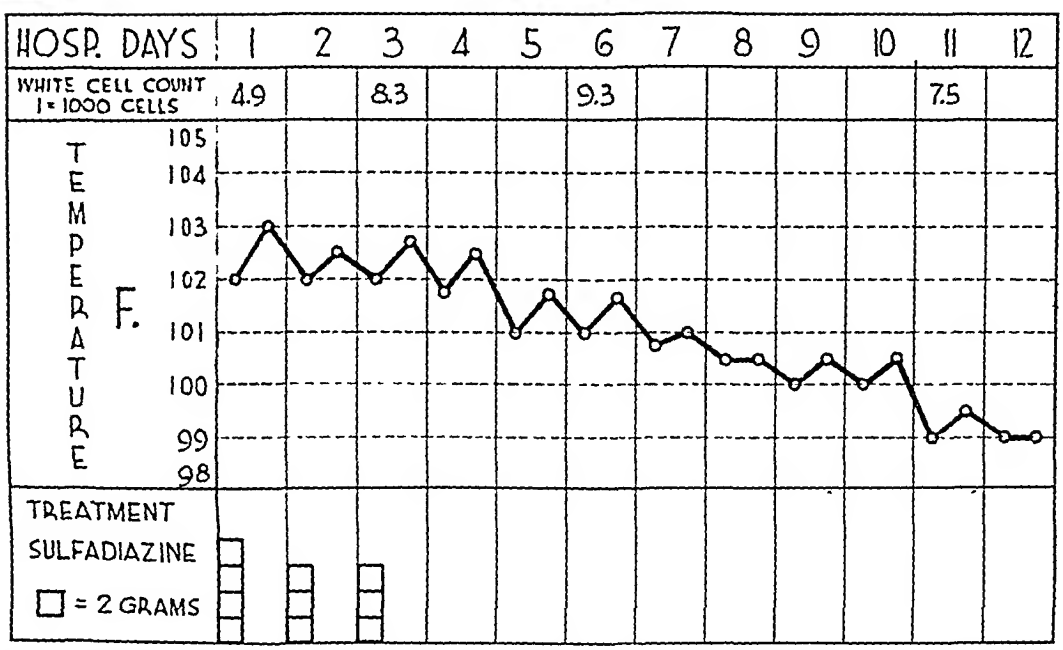
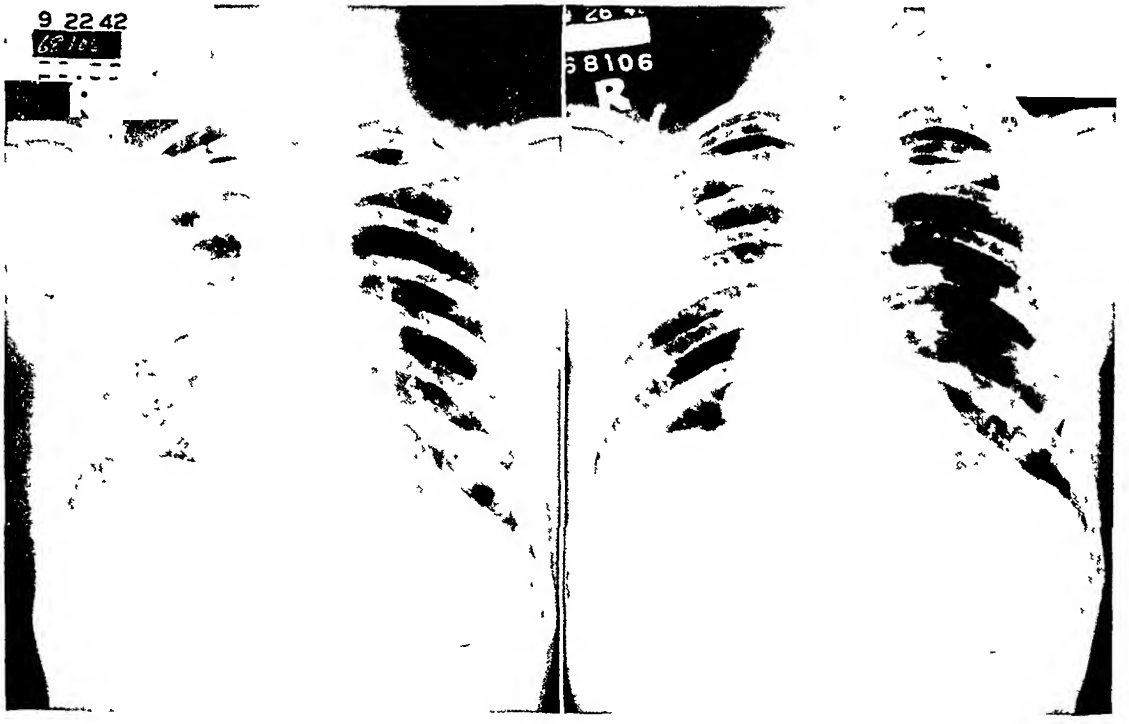


FIG. 1 represents a composite chart of Case 1 and roentgen films revealing a lesion of lower lobe on June 23, 1942; with slow resolution and residual pleuritis on July 15, 1942.

On admission his temperature was 103 degrees, pulse 100, respirations 25. He was placed on sulfadiazine for seventy-two hours, but there was no response and

the drug was discontinued. He ran a self-limited, slightly prolonged but uneventful course and was discharged after hospitalization of one month. This case is an example of atypical pneumonia, cause undetermined, with a leukopenia and a poor response to chemotherapy.

Case 2. A 32-year-old, black male, Panamanian, laborer, was admitted to Gorgas Hospital on September 21, 1942. He was ill for four days with chills, fever, upper respiratory symptoms and a slightly productive cough. The only positive objective signs were fine inspiratory rales in the upper right axilla and at the level of the second anterior interspace. The white cell count was 16,000 on admission with a differential of 88% polymorphonuclear cells and 12% lymphocytes. Sputum cultures were negative on three occasions and



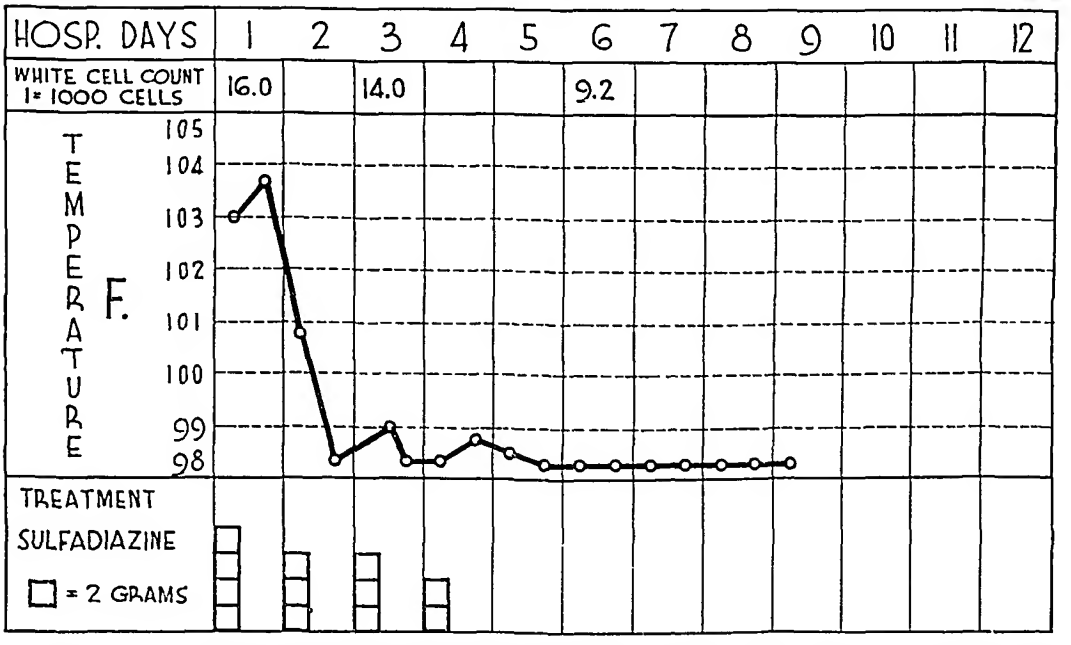


FIG. 2 represents a composite chart of Case 2 and roentgen films revealing a lesion of right upper lobe on September 22, 1942, and marked resolution on September 26, 1942.

productive cough. Two days prior to admission he had moderate hemoptysis and his cough became productive. His initial white cell count was 9,300 with a differential of 78% polymorphonuclear cells and 22% lymphocytes. Sputum cultures on several occasions were negative and daily concentrated smears for acid-fast bacilli were negative. X-rays (see fig. 3) of July 2, 1942 revealed pneumonitis of the right upper lobe with a suggestive area of rarefaction. On July 8, 1942 there was marked resolution of the lesion and a roentgen film of July 18, 1942 was negative.

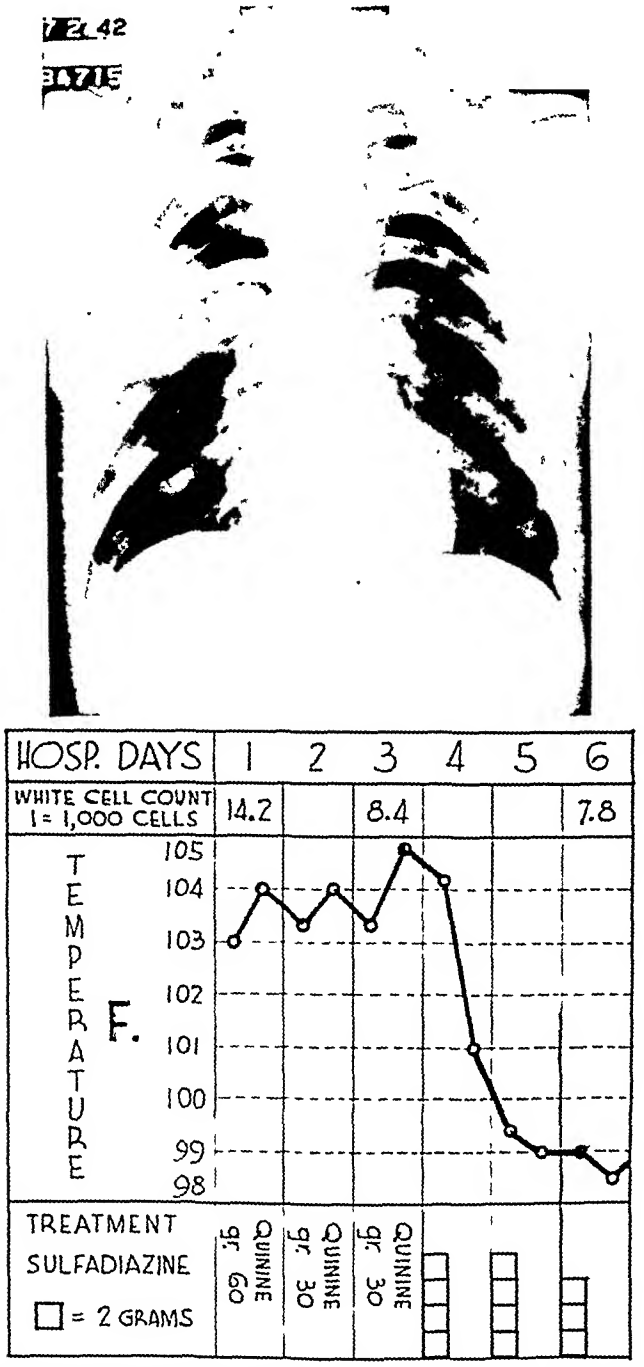


FIG. 4 represents a composite chart of Case 5 and a roentgen film revealing a pneumonitis of the right upper lobe on July 27, 1942.

Tuberculosis was suspected because of the history and the location and type of roentgen shadow. The treatment was palliative and the course in the hospital uneventful with gradual clearing of the parenchyma of the lung. This case indicates the importance of serial

films in eliminating the diagnosis of tuberculosis which was an early important consideration.

Case 4. A 40-year-old black, laborer, Panamanian, was admitted to the hospital on July 26, 1942. He was ill for two days with chills, fever, and headache. There was no respiratory symptoms. On admission there were no abnormal physical signs in the chest. A few days later fine inspiratory rales and broncho-vesicular breath sounds were noted over the midaxillary and lower portions of the right upper lobe. Malarial smears and routine workup were negative. His white cell count was 14,200 with a differential of 88% polymorphonuclear cells and 12% lymphocytes. As malaria was suspected he was placed on quinine treatment, but there was no response. Subsequent thick and thin smears were negative for plasmodia. On July 29, 1942 he started to cough and expectorate and the chest film of July 27, 1942 indicated a lesion of the right upper lobe (see fig. 4). He was placed on sulfadiazine and an immediate response was noted. In two successive daily sputum cultures *Streptococcus hemolyticus* was identified. His course was then uneventful and short. This is an example of a case suspected of malaria with a poor response to anti-malarial drugs; and the subsequent uncovering of a bacterial pneumonitis with excellent sulfonamide effect.

COMMENT

The epidemological aspects of pneumonias in Panama are worthy of study. As it is believed that the disease is chiefly transmissible from patient to patient, the congregation of large masses of individuals in army and labor camps in this zone offers a particular hazard in all types of pneumonias. Respiratory secretions via the atmosphere are the chief vehicles, and in numerous instances such a modus operandi had been observed on the wards where isolation technique was not employed. It is believed, as has been stated by Dingle et al. (18), that unrecognized infections which may constitute the effective source of spread of the disease exist in various camps. Therefore, the employment of "sick-call" for mild colds and respiratory infections is an important method of control, and much responsibility is placed on the medical corps in eradicating this problem.

Other possibilities undoubtedly exist and deserve investigation. There has been no suggestion that food or water are factors; nor can insects or other forms of animal life, common in Panama, be indicated on the basis of existing evidence in suspected virus infections. However, it has been demonstrated elsewhere that atypical virus pneumonia may be spread from man to cats, rats, birds and vice versa (19). Because of the relatively common occurrence of pneumonia in malaria, one wonders whether the same *Anopheline* mosquito can be incriminated as a vector of a pneumonitis-producing virus. *Aëdes aegypti* mosquitoes, ticks, sandflies and related insects, which are common in this area, are also to be considered.

In observing the results of bacteriological studies of the sputum, one is impressed with the large percentage of negative cultures. Although a number of these cases were not characteristic of atypical pneumonia in all respects, as it has been described in the literature by numerous contributors (20), it is felt that the majority of cases fall into this category. Contrary to accepted teaching, 32% was accompanied by a leukocytosis, 13+% had a lobar distribution, and 34% exhibited a very satisfactory response to chemotherapy. As many cases were admitted to the hospital comparatively late in the disease, it is quite possible that secondary bacterial infection influenced the hematological picture and the response to sulfonamides. Those cases with leukocytosis were particularly susceptible in a favorable manner to the sulfa drugs. The figures of 32% leukocytosis and 34% good therapeutic response appear to be more than coincidental and suggest that chemotherapy is of special value in cases with a polymorphonuclear trend. In many respects, however, the illness ran a classical course as to onset, systemic manifestations, paucity of objective findings, and severity. Complications were rare and the prognosis usually excellent. All of these factors conform with the clinical concept of the disease now termed "atypical pneumonia, cause undetermined."

Comparing the bacterial group with the negative sputum culture group, certain fundamental differences are noteworthy. In the former series the onset was acute in a larger percentage of cases (71%:36%), absence of abnormal physical signs was less common (14%:28%), lobar distribution was more frequent (23%:13%), leukocytosis was more prevalent (74%:32%), and the majority of cases exhibited an adequate response (74%:34%) to sulfonamides.

Facilities for virus study were not available in this series, but it is to be noted that investigations elsewhere have produced a very small percentage of positive results. In various instances the viruses of influenza, choriolymphocytic meningitis, lymphogranuloma venereum, meningo-pneumonitis, psittacosis, and *Rickettsia burneti* of Q fever have

been suspected of producing a similar pulmonary picture. In several instances, when a specific virus was not identified, the disease was transmitted to such experimental animals as the mongoose (21) and Java Rice birds (22). At present the average institution is unable to carry out extensive investigations in this field. Such tests as the study of cold agglutinins (23) appear promising, but certainly are without specificity.

Special consideration must be given in tropical zones to the possibility of secondary involvement. The pulmonary inflammation may be part of a protozoal disease, and such lesions have been described in kala-azar, amebiasis and malaria. Pneumonia also has been described in such metazoal diseases as ascariasis, strongyloides infestation, ankylostomiasis, and fluke infections. In Panama, where the humidity is great and fungi thrive, the mycoses are also to be differentiated from the usual types of pneumonitis. Monilia, torula, coccidioides, blastomyces may be mentioned as possible factors. Pulmonary tuberculosis, a prevalent disease in Central America, is always to be ruled out, especially in upper lobe lesions; and serial roentgen studies are of considerable aid.

The problem of control can be mastered to a reasonable extent by general measures, which Keefer (24) has enumerated. The medical profession and public should be educated concerning the dangers of spread by contact. Isolation of all active cases, adequate medical care of colds and bronchitis, destruction of all respiratory discharges and early recognition of the disease are steps in the right direction. A prompt trial of chemotherapeutic agents is often indicated. The avoidance of overcrowding, the betterment of hygienic conditions are other worthwhile general measures. The institution of these preventive methods is the duty of the medical corps of the military services and the health organizations of the communities.

1. A study of 500 consecutive cases of primary pneumonia admitted to Gorgas Hospital during an 18-month period (1942–1943) was completed.

2. In addition to other laboratory procedures, repeated sputum specimens and serial roentgen films were studied.

3. On the basis of these studies, the following classification of this series was submitted:

	No. of Cases	%
a. *Negative sputum culture group.* Most of these cases resembled the disease designated as "atypical pneumonia, cause undetermined."	359	71+
b. *Bacterial group*	141	28+
(1) Pneumococcus, various types	72	
(2) Streptococcus hemolyticus	31	
(3) Pneumococcus, untyped	30	
(4) Streptococcus anhemolyticus	8	

4. The bacterial group differed from the negative culture group in exhibiting a higher percentage of acute onset, abnormal physical signs, lobar distribution, leukocytosis and adequate therapeutic response to sulfonamides.

5. The clinical course in each group was relatively benign and complications were few. There was one death in the negative culture series (mortality rate of 0.28%) and there were three deaths in the bacterial series (mortality rate of 2.1%).

6. At the onset malaria was an important consideration in differential diagnosis and approximately 10% of all patients received anti-malarial therapy. In upper lobe lesions, particularly when resolution was delayed, tuberculosis had to be ruled out.

7. Several illustrative case reports and a brief summary of the deaths were presented.

8. In most instances the problem of pneumonia in Panama is not different than elsewhere. However special consideration must be given to non-bacterial agents (metazoal, protozoal) in tropical areas.

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RISK OF ATTACK IN LEPROSY IN RELATION TO AGE AT EXPOSURE

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INTRODUCTION

It is a common opinion among leprologists that the risk of developing leprosy is affected predominantly by the age at which exposure takes place. Recently, this opinion has been expressed clearly by one of the leading students of the disease, Dr. R. G. Cochrane, as follows: "The greatest individual cause of infection is close contact, . . . this and the age of the individual play a far greater part in the epidemiology of leprosy than all the other factors frequently suggested" (1). It is on this conception that the practice of prompt segregation of patients with lepromatous leprosy and the policy of early removal of infants from contact with leprous parents are based.

There is now sufficient evidence that the risk of contracting leprosy is far greater for individuals exposed to lepromatous patients in the household than for other persons living in the same community. In the Philippines the relative risks are five or six to one (2). Also, for the same area (2) statistical evidence shows that the average age at onset is somewhat younger for patients known to have been subjected to household exposure than for others, although in both exposed and nonexposed the highest incidence occurs in the age group 10 to 15 years.

More precise measurement of the influence of age at exposure is obviously desirable but very detailed epidemiological studies are necessary for this purpose. The total number exposed must be determined, a fact obtainable only for households in which a case has occurred. It is necessary to learn also the type of leprosy, both for primary and secondary cases, and, as closely as possible, the dates of onset. In addition, dates of birth, and subsequent histories through a sufficient period of years, must be ascertained for all members of these households.

For milder forms of leprosy, incidence can be measured accurately only by keeping such individuals under close supervision for a long time. For lepromatous cases, it is, however, quite possible to estimate rates of incidence from the study of the past experience of existent households.

SOURCE OF DATA

Data for the present analysis were collected in 1933 and in 1936–7 in two municipalities in the Philippine Islands, Cordova and Talisay, in the province of Cebu. Surveys were under the joint sponsorship of the Bureau of Health of the Philippines and the Leonard Wood Memorial Fund.

Cordova, the first area studied, is situated on the small island of Mactan about one mile east of the city of Cebu. The island is low-lying and of coral formation. At the time of the survey few crops were grown and the population was economically dependent on fish, copra, and hemp which is derived from the maguey plant. The general economic condition of the municipality was very poor.

Talisay, on the main island of Cebu, is situated about 7 miles south of the city of Cebu. The area surveyed included not only the low-lying plains but also a river valley and hills. Unlike Cordova, the soil is relatively fertile, the principal crop being sugar cane. At the time of the survey some fishing was done and in most areas fruit and vegetables were grown. The general economic level of the population was appreciably above that of Cordova.

The procedures which were followed in both communities included (1) a house-to-house census of the inhabitants, (2) physical examination of the inhabitants for leprosy and other skin diseases, and (3) epidemiological investigation of all cases of leprosy. Cooperation of the population was excellent; 99.3 per cent of 10,672 inhabitants in the Talisay area and 98.3 per cent of 6,063 in Cordova, submitted to examination.

The two municipalities were alike in that each

[1] These studies have received support from the American Leprosy Foundation, the Bureau of Health of the Philippines, and Western Reserve University. Drs. Guinto and Rodriguez did not have an opportunity to read this manuscript: both are interned in the Philippines.

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had a relatively high prevalence[2] of leprosy (3, 4). In Cordova in 1933, the prevalence rate was 17.2 per 1,000, but exclusion of neural cases considered to be quiescent or arrested, lowered the rate to 13.4. In Talisay in 1936–7, the total rate was 19.5, reduced to 15.2 by exclusion of inactive neural cases. In Cordova 44.2 per cent of the patients were known to be bacteriologically positive and in Talisay 50 per cent. The prevalence in both areas was higher in males than in females.

The epidemiological investigation included for each household the complete family roster, with birthdate, sex, and leprosy status for all past and present members. For exposed persons not present in the household the date of death was ascertained, or the date of departure, if moved to another residence. The date of onset and type of leprosy were determined for each patient with leprosy. Information obtained from the family was checked against the records of the Culion Leprosarium and of the Eversley Child's Treatment Station at Cebu.

The data relating to influence of age at exposure were considered to be sufficiently complete only for the study of lepromatous leprosy; that is, the analysis is restricted to records of individuals exposed to lepromatous cases in the household and the secondary cases included in the study are only those of the lepromatous type.

The families included are those for which it was possible to complete the life experience of every known member from entrance into the family, either to death, to development of leprosy, to departure to another household or community, or to the end of the period of observation, taken as June 30, 1933, for Cordova and as October 30, 1936, for Talisay.

METHOD OF ANALYSIS

To determine the risk of developing leprosy a modified life-table method was adopted. This method involves determination of person-years of risk, that is, each year of life of an individual is regarded as a unit. For any given group the incidence rate is expressed as the average number of cases per 1,000 person-years, which is regarded as equivalent to observation of 1,000 persons for one year. The schedules include individuals who where born eighty years or more before the date of investigation and infants born only a month or two before. It should be borne in mind, therefore, that the incidence rates are not applicable to the present but are a statement of what has occurred during the life experience of households of Cordova and Talisay, in which one or more members were living and present at the time of the inquiry.

An individual was considered to have been exposed if he had lived under the same roof as a leprous person for a period of at least one month. Subsequently he was considered to be at risk as long as he remained in the community, regardless of the removal of the patient from the household or of the removal of the individual to another residence in the community.

Individuals born in any household in which there was an existent case of leprosy were considered to be at risk from birth. Those present at the time of onset of the primary case were allotted one-half year of risk at their age at time of onset. Individuals entering the household while a leprous person was present were likewise given one-half year's experience at their age at entrance. Departures, either because of death or removal, were given one-half year's experience at their age at time of departure.

Since previous analysis had shown that the two areas were similar as regards both prevalence rates and incidence rates, data from Cordova and Talisay were combined. On the family schedules there were included the records of 755 males and 765 females, who gave a history of exposure in accordance with the definition adopted. For each of these individuals the years of risk were calculated, yielding a total of 19,553 person-years. In this experience there occurred 89 secondary cases of lepromatous leprosy, or an average annual incidence of 4.6 per 1,000 person-years. It may be noted that this is approximately 5.5 times the incidence rate for persons in the general population not known to have been subjected to household exposure.

INFLUENCE OF AGE AT EXPOSURE

The life experience of these households, classified according to the ages at which the individuals were first exposed, the cases of lepromatous leprosy developing, and the incidence rates, are given in table 1, by sex and age.

This table indicates clearly a definite relationship between age at exposure and age at which signs of leprosy were first observed. Among chil-

[2] By prevalence is meant the number of cases existent in a community on a specified date. In contrast, incidence refers to the number of new cases occurring in a specified period of time.

dren who were exposed at ages under 5 years, the majority at birth, no cases occurred before they reached 5 years of age. Lepromatous cases have been reported in young children but are rare (5); most have been of the neural (macular) type.

TABLE 1

Secondary attack rates for lepromatous leprosy following household exposure to lepromatous leprosy, according to age at exposure and age at attack

Cordova and Talisay, Cebu, Philippine Islands

AGE AT EXPOSURE	SEX	AGE AT ATTACK: Under 5 years			5–9 years			10–14 years			15–19 years			20 years and over			All ages			RATIO OF RATE TO RATE AT ALL AGES
		Person-yrs.	No. of cases	Rate per 1000	Person-yrs.	No. of cases	Rate per 1000	Person-yrs.	No. of Cases	Rate per 1000	Person-yrs.	No. of cases	Rate per 1000	Person-yrs.	No. of cases	Rate per 1000	Person-yrs.	No. of cases	Rate per 1000	
years Under 5	Male	955	0	0.0	983	12	12.2	699	17	24.3	453	7	15.5	497	2	4.0	3587	38	10.6	1.6
	Female	812	0	0.0	781	2	2.6	540	5	9.3	354	3	8.5	349	1	2.9	2836	11	3.9	1.6
Total.....		1767	0	0.0	1764	14	7.9	1239	22	17.8	807	10	12.4	846	3	3.5	6423	49	7.6	1.7
5–9	Male				245	0	0.0	383	7	18.3	233	3	12.9	284	1	3.5	1145	11	9.6	1.4
	Female				214	0	0.0	372	0	0.0	254	3	11.8	317	1	3.2	1157	4	3.5	1.4
Total.....					459	0	0.0	755	7	9.3	487	6	12.3	601	2	3.3	2302	15	6.5	1.4
10–14	Male							205	1	4.9	338	1	3.0	477	5	10.5	1020	7	6.9	1.0
	Female							224	2	8.9	333	1	3.0	504	0	0.6	1061	3	2.8	1.1
Total.....								429	3	7.0	671	2	3.0	981	5	5.1	2081	10	4.8	1.0
15–19	Male										161	2	12.4	738	2	2.7	899	4	4.4	0.7
	Female										178	0	0.0	945	2	2.1	1123	2	1.8	0.7
Total.....											339	2	5.9	1683	4	2.4	2022	6	3.0	0.7
20 and over	Male													2916	4	1.4	2916	4	1.4	0.2
	Female													3809	5	1.3	3809	5	1.3	0.5
Total.....														6725	9	1.3	6725	9	1.3	0.3
All ages	Male	955	0	0.0	1228	12	9.8	1287	25	19.4	1185	13	11.0	4912	14	2.9	9567	64	6.7	
	Female	812	0	0.0	995	2	2.0	1136	7	6.2	1119	7	6.3	5924	9	1.5	9986	25	2.5	
Total.....		1767	0	0.0	2223	14	6.3	2423	32	13.2	2304	20	8.7	10836	23	2.1	19553	89	4.6	

Between 5 and 10 years of age the annual incidence rate averaged 7.9 per 1,000. The rate increased to a maximum of 17.8 at 10 to 15 years and fell to 12.4 at 15 to 20 years. The rate for persons 20 years and over who were exposed before 5 years of age was only 3.5 per 1,000. Thus the experience, at successive ages, of those exposed in infancy and early childhood in these households, shows that the highest incidence of lepromatous leprosy is reached about 10 years after exposure.

Turning to those exposed at 5 to 10 years of age, no cases occurred before the age of 10 years. Between 10 and 15 years the incidence rate was 9.3 per 1,000, increasing to a maximum of 12.3 at 15 to 20 years, and falling off sharply to 3.3 at 20 years of age and over. Again, the age of maximum incidence occurred 10 years after exposure.

For those exposed at 10 to 15 years, the maxi-

mum incidence rate, 7.0 per 1,000, occurred in this same age group. The rate fell to 3.0 at 15 to 20 years and increased to 5.1 at 20 years and over.

Similarly for those exposed at 15 to 20 years the maximum incidence rate, 5.9 per 1,000 again occurred in the same age group. The rate fell to 2.4 for those of 20 years and over.

These data indicate a variable period between exposure and development of noticeable lesions of lepromatous leprosy. Taking the statistics at their face value, this period must be much longer when exposure occurs in infancy and early childhood than when it occurs in later childhood and adolescence. The average interval between exposure and recognition of the disease in 64 persons exposed before the age of 10 years was 10.5 years; and for 25 individuals exposed after the age of 10 years, it was only 6.0 years. It may be that some secondary factor more common to adolescents than to young children is necessary to bring a latent infection to light.

These data also reveal a definite relationship between the age at which exposure takes place and the probability of developing leprosy. The average annual incidence rate for those exposed before 5 years of age was 7.6 per 1,000; for those exposed between 5 and 10 years, 6.5; for those exposed between 10 and 15 years, 4.8; for those exposed between 15 and 20 years, 3.0; and for those exposed at ages over 20 years, it was only 1.3 per 1,000. The ratio of the attack rate for each group to the rate for all ages thus decreases regularly, for each age band at exposure, from 1.7 to 1 for those exposed before 5 years of age to 0.3 to 1 for those exposed after the age of 20 years. This suggests greater susceptibility of young children to the disease; the earlier the exposure the greater the risk. In available records there are insufficient data to exclude the possibility that some or all of the excess incidence observed in those exposed at the younger ages may be attributable to greater intimacy of exposure. An infant crawls about, handles contaminated objects, and may be fondled by a leprous member of the family. The comment may be made, however, that in these households, the primary case occurred infrequently in the mother.

Nothing has been said regarding the possible effect of duration of exposure. In the Philippines, for many years, lepromatous cases have been segregated promptly following discovery. It is not likely that the period between onset and discovery would be affected by the ages of those exposed in the household.

Examining the data for males and females separately it will be seen that the major fact previously noted is true for both sexes, namely, there is positive correlation between age at exposure and risk of contracting leprosy. This risk is very much higher for males than for females regardless of age at exposure. From other studies it would appear that this finding may be peculiar to lepromatous leprosy. Incidence rates for neural leprosy do not indicate a pronounced selectivity for either sex (2). The marked difference between incidence rates for the sexes in early childhood, and especially for those exposed at birth or shortly after, leads to a first assumption that the responsible factor is not greater exposure of males than of females but rather greater susceptibility of males.

Judging from the experience of those exposed under 5 years, admittedly too limited an experience to justify a final conclusion, it would appear that if a difference in susceptibility between the sexes exists, it tends to decrease as adult life is approached. For children in successive quinquennial age groups from 5 to 9 years upwards, the ratio of male to female incidence was 4.7, 2.6 and 1.8 to 1. For those 20 years and over, it was 1.4 to 1. This decreasing ratio is difficult to explain, assuming about the same number of males as of females to be infected, some secondary factor must reduce the resistance of the former. This factor whether it be an inherent susceptibility as we have previously suggested (2), or some external factor, becomes progressively less important as the children grow older.

In reaching the conclusion that greater susceptibility of the male is the chief factor, it has been assumed that environmental conditions are identical for male and female children. It should be mentioned, however, that in the localities under consideration, there was a conspicuous tendency to provide small female children with clothing to a greater extent than male. This would be protective if *Mycobacterium leprae* enters through the skin.

In favor of the view that males are more susceptible than females is the fact that the overall picture, when exposure to the disease takes place in later childhood, also indicates a substantially greater risk for males. Unfortunately the data are too limited to justify further speculation on this matter.

SUMMARY

A study of the life experience of 1520 individuals living in the province of Cebu, P. I., and exposed

in the household to lepromatous leprosy reveals the following facts:

1. There is a clear relationship between age at time of exposure and the risk of developing lepromatous leprosy. It is highest for those exposed before the age of 5 years, decreasing progressively as the age at time of exposure increases. This is true for both males and females.

2. The ratio of the incidence rates for males to those for females, for those exposed before 5 years of age, varies from 4.7:1 for rates in the age band of 5 to 10 years to 1.4:1 for those of 20 years and over.

3. The average interval between exposure and development of lepromatous leprosy was 10.5 years for those exposed under 10 years of age but only 6.0 years for those exposed after the age of 10 years.

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TREATMENT OF LAMBLIASIS WITH ACRANIL[1]

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The purpose of this paper is to report on the treatment of *Giardia lamblia* infection carried out by us during 1938–1939 with "acranil" (3 chloro-7-methoxy-9-(2 hydroxy-3-diethyl-amino) propyl-amino-acridine-dihydrochloride), an acridine derivative closely related to "atabrine" or "quinacrine." This study was carried out with a view of determining the effect of the drug on infection with *G. lamblia*. No attempt was made to study the symptomatology or pathogenesis of lambliasis nor to evaluate the therapeutic effect of the drug on the clinical syndrome evoked by the infection.

Moore and Dennis (1940) fully discussed the incidence and importance of *Giardia lamblia* in diarrheas of children at Beirut, Lebanon. Breuer (1938) gave a full discussion of the parasitology, epidemiology, pathogenesis, symptomatology and prophylaxis of lambliasis and reviewed the effectiveness of most of the drugs used for treatment of this condition up to 1938. We do not feel justified in presenting a second review of the same subject. We fully endorse the conclusion reached by Breuer (1938) and by Carrière and Huvèz (1932), that previously described and "recognized" methods of treatment of lambliasis are deceptive and generally unsatisfactory. With the discovery of a selective chemotherapeutic effect by "atabrine" or "quinacrine," the treatment of lambliasis entered a new phase and is receiving the increased attention which it deserves.

Galli-Valerio (1936) was the first to report the effect of "atabrine" on *Giardia lamblia* infection in man. He and his pupils found a 40% infestation with *G. lamblia* in school children in Tessin, Switzerland. They observed that two of the children who had taken atabrine became free of the parasites and decided to study the effect of atabrine on *G. lamblia*. A few months later, Martin (1936) used "quinacrine" (the French equivalent of German "atebrin") and reported favorable results obtained in 51 of 54 cases thus treated. Martin gave a single course of treatment with a daily dose of 0.3 gm. of quinacrine for five successive days. Tanguy (1937) successfully treated 19 cases with quinacrine, using daily doses of 0.3 gm. for five days, followed by a second course of treatment given five days after termination of the first series. In only one case was it necessary to give a third and a fourth treatment before final cure. Lucien Brumpt (1937) found quinacrine to be highly effective against *Giardia muris* infection in mice. Tecon (1937) confirmed Martin's findings. Pagniez (1937) reported the successful treatment, with quinacrine, of a patient who had resisted previous treatment with arsenicals and other therapeutic agents.

Fagot (1938), Garin (1938), Grüneis (1938), Heilman (1938), Bacigalupo (1939), Morrison and Swalm (1939) and many others have reported favorably on the treatment of lambliasis with atabrine or quinacrine. Grott (1938–1939) reported favorably on the treatment of lambliasis with atabrine, with sostol and with acranil.

Although *Giardia lamblia* is one of the most common intestinal flagellates found in man, its incidence varies with country, locality and age. It is most common in children in communities where the incidence of protozoal infections in general is highest. The infestation rate is reported to vary between 1.66–48.7%. The significance and size of the problem is emphasized by Moore and Dennis (1940) and the data presented in table 1.

EXPERIMENTAL

The cases studied were selected from amongst three hundred children in two orphanages. The stools of these children were at first carefully examined for all kinds of intestinal protozoa and helminths by the following three methods:

a. Direct smear method.
b. Hydrochloric acid and ether concentration method, for helminth ova.
c. Rectal swab in accordance with Hall's technic (1937).

Apart from *Giardia lamblia*, we found that these children were heavily infested with other intestinal protozoa and helminths (table 2).

[1] Acranil was provided for these experiments by the manufacturer, "Bayer," Leverkusen.

The high rate of infestation with *Enterobius vermicularis* (54.7%) was demonstrated by the use of Hall's cellophane swab procedure (1937); the infestation rate was only 1% by the direct smear and concentration methods. The effect of acranil on the fifty cases of lambliasis has been studied. These cases were also heavily infected with other protozoa and helminths (table 3).

As it is well-known that the encystment of *Giardia lamblia* occurs intermittently, enormous numbers of cysts appearing in a short time and then none for several days, followed by a shower of cysts later, it was decided to make five weekly stool examinations, beginning one week after the administration of the drug. These fifty cases were divided as follows:

7 children 3–6 years old, received 0.1 gm. of acranil a day, given in a single dose for five consecutive days.

25 children between the ages of 7–12 received 0.2 gm. of acranil a day in 0.1 gm. doses, given morning and evening after meals, for five consecutive days.

10 children 13 years or more in age were given 0.3 gm. of acranil a day in 0.1 gm. doses, three times daily for five consecutive days.

2 children, 7 years old, with *Giardia lamblia* and *Hymenolepis nana* infestation were given 0.2 gm. acranil in one dose on the first day, early in the morning, followed by 0.1 gm. a day after breakfast for three more days.

TABLE 1

Reported incidence of G. lamblia infection of man in different parts of the world

AUTHOR	LOCALITY	NUMBER	% INFECTION
Moore and Dennis (1940)	Beirut, Lebanon (in diarrheas of children age (1–12)	543	15.5
Lund & Dennis (1939)	Lebanon & Syria (population survey)	1465	10.02
Dennis & Lund (1937)	Hospital and clinic patients, Beirut	4234	5.48
Senekji, Boswell & Beattie (1939)	Iraq	1000	8.5
Boeck & Stiles (1923)	U.S.P.H.S. Hospitals	8029	6.5
Williamson, Kaplan & Geiger (1929)	Chicago food handlers, Chicago hospital and	1148	6.53
	dispensary cases	380	1.66
Faust (1929)	North China	13617	1.77
Meleney, Bishop & Leathers (1932)	Tennessee, U.S.A.	20237	14.7
Kessel & Mason (1930)	Los Angeles General Hospital	2731	4.4
Andrews & Paulson	Hospital outpatients, Baltimore	312	3.5
Summerlin (1934)	Adult private patients, San Diego	1339	2.4
	Children	513	3.7
Andrews (1934)	Fresnillo, Mexico	2303	5.0
Hill, C. M. & Hill, R. B. (1927)	Porto Rico—children	125	48.7
Ter-Mateossian	Armenian (U.S.S.R.)	1200	8.2–24
Bacigalupo (1939)	Buenos Aires, Argentine	7656	7.45

TABLE 2

Results of examination of stools of 300 children

PROTOZOAL INFECTIONS			HELMINTHIC INFESTATIONS		
Protozoa	No.	%	Helminths	No.	%
Endamoeba histolytica	31	10	*Ascaris lumbricoides*	27	9
E. coli	123	40	*Trichuris trichiura*	43	14.3
Endolimax nana	64	21	*Enterobius vermicularis*	163	54.7
Iodamoeba williamsi	17	5.6	*Taenia saginata*	27	9
Chilomastix mesnilli	4	1.3	*Hymenolepis nana*	27	9
Giardia lamblia	50	16.6			

1 child, ten years old, also infected with *G. lamblia* and *H. nana* was given 0.4 gm. of acranil on the first day on empty stomach, and thereafter was given 0.2 gm. a day in 0.1 gm. doses morning and evening for three more consecutive days.

2 children, 16 years old, infected with *G. lamblia* and *H. nana* received 0.5 gm. of acranil on an empty stomach on the first day, and then 0.3 gm. daily in 0.1 gm. doses, three times a day for three consecutive days.

3 adults with *G. lamblia* and *Taenia saginata* infection, received 0.5 gm. acranil on an empty stomach on the first day, and then 0.3 gm. in 0.1 gm. doses three times a day for three more consecutive days.

These data are summarized in table 4.

TABLE 3

Protozoa and helminths associated with Giardia lamblia in the 50 cases under study

PROTOZOA	NO.	%	HELMINTHS	NO.	%
Endamoeba histolytica	10	20	*Ascaris lumbricoides*	6	12
E. coli	17	34	*Trichuris trichiura*	10	20
Endolimax nana	15	30	*Enterobius vermicularis*	18	36
Iodamoeba williamsi	6	12	*Taenia saginata*	3	6
Chilomastix mesnilii	2	4	*Hymenolepis nana*	5	10

Children suffering from *G. lamblia* infection alone, were given 0.05–0.2 gm. calomel, according to their age, on the evening before beginning treatment with acranil. The tablets of acranil were given after meals as described above. No attempt was made to modify the diet of these children. The children thus treated presented no ill effects from treatment other than the yellow discoloration of their skin. Those children who were infected with *G. lamblia* and *H. nana* or *T. saginata* were treated differently in that they were given a calomel laxative the night before treatment. The next morning, on an empty stomach, they were given 2–5, 0.1 gm. tablets of acranil as described above, and three hours later a sodium sulfate purge was administered. Food was given three hours after the purgative. Three out of eight children treated in this way vomited their acranil tablets shortly after swallowing them, and they were given a second dose. The day following the treatment for tapeworms, acranil treatment for *Giardia lamblia* infection was continued for three successive days as described.

TABLE 4

Results of treatment of 50 cases of lambliasis with acranil

AGE GROUP	NO. OF CASES	FINDINGS BEFORE TREATMENT	TREATMENT WITH ACRANIL	5 WEEKLY EXAMINATIONS FOR G. LAMBLIA AFTER TREATMENT
3–6 years.....	7	*G. lamblia* only	0.1 gm. daily for 5 days	Negative
7–12 years.....	25	*G. lamblia* only	0.2 gm. daily for 5 days	Negative
13 and more.....	10	*G. lamblia* only	0.3 gm. daily for 5 days	Negative
7 years.....	2	*G. lamblia* + *H. nana*	0.2 gm. on 1st day, then 0.1 gm. for 3 days	Negative
10 years.....	1	*G. lamblia* + *H. nana*	0.5 gm. on 1st day, then 0.3 gm. for 3 days	Negative
Adults.....	3	*G. lamblia* + *T. saginata*	0.5 gm. on 1st day, then 0.3 gm. for 3 days	Negative

Beginning one week after the administration of the last dose of acranil, weekly stool examinations were made for five consecutive weeks. All of the examinations for *Giardia lamblia* were negative. Hence it can be safely concluded that acranil is an efficient chemotherapeutic agent for the treatment of lambliasis in man.

Acranil was found to have no parasiticidal effect on the concomitant protozoal infections with *Endamoeba histolytica, E. coli, Iodamoeba williamsi, Endolimax nana, Chilomastix mesnilii,* or on nematode infestations with *Ascaris lumbricoides, Trichuris trichiura* and *Enterobius vermicularis.*

CONCLUSION

The therapeutic effect of "acranil," an acridine derivative closely related to atabrine or quinacrine, was tested on fifty cases of *Giardia lamblia* infec-

tion in children. Acranil was found to be a highly efficient chemotherapeutic agent for lambliasis as shown by five consecutive weekly negative stool examinations made on all of the cases after medication. The selective chemotherapeutic index of the drug may thus be considered as 100%. The drug was very well tolerated by the children, none of whom showed any toxic effects. There was slight discoloration of the skin which gradually disappeared.

Acranil was found to have no parasiticidal effect on concomitant protozoal infections with *Endamoeba histolytica*, *E. coli*, *Iodamoeba williamsi*, *Endolimax nana* and *Chilomastix mesnilii*, or on nematode infestations with *Ascaris lumbricoides*, *Trichuris trichuria* and *Enterobius vermicularis*. Acranil was found to have some therapeutic effect against cestode infestations with *Hymenolepis nana* and *Taenia saginata*. The results of the treatment of these tapeworm infestations with acranil will be given in a separate communication.

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Knowles's Introduction to Medical Protozoology. Second Edition. By B. M. DAS GUPTA, Professor of Protozoology and Director, Calcutta School of Tropical Medicine. 104 illustrations and 10 coloured plates. P. I, VIII, 1–323. U. N. Dhur & Sons, Ltd., Calcutta, 1944.

This interesting book is not presented as a comprehensive substitute for the more complete reference books of protozoology but it is intended as a teaching text for advanced students of this subject. It reads easily and the material is well organized. The colored plates are very clear and well arranged. The binding is not very substantial.

Greatest emphasis in content is devoted to the Rhizopoda (Entamoeba), the Sporozoa, (Leishmania and Trypanosoma) and to the Sporozoa (Plasmodia), as is commensurate with their incidence. Attention is also directed to the spirochaetes and certain parasites of doubtful nature such as Toxoplasma, Bartonella, Rickettsia, Anaplasma, etc. There are excellent lists of pertinent references at the end of each chapter, along with appendices for similar organisms occurring among the lower animals. One limitation to its use here in the United States is the reference to some chemotherapeutic agents which are unavailable in this country such as Bayer "205" for trypanosomiasis. However, in this respect little emphasis is placed on therapeutic measures throughout the book. Clinical manifestations of the diseases are not described very extensively.

This book can be recommended for students because of the details given to certain diagnostic procedures such as splenic and sternal punctures, and laboratory methods for staining, culturing and mounting specimens. Attention is given to the distribution and occurrence of the protozological diseases in India, an area of the world in which they are of very great significance.

DONALD J. WILSON.

Manual of Clinical Mycology. By NORMAN F. CONANT, PH.D., DONALD STOVER MARTIN, M.D., DAVID TILLERSON SMITH, M.D., ROGER DENIE BAKER, M.D., AND JASPER LAMAR CALLAWAY, M.D. Pp. I-378, Illustrated. W. B. Saunders and Co., Philadelphia and London.

Under the auspices of the division of medical science of the National Research Council, five members of Duke University's Medical faculty have collaborated in the preparation of a new manual of medical mycology. This latest publication in a series of military medical manuals is designed primarily to meet the need of military medical officers serving in the tropics of the South Pacific or South America where mycotic infections are commonly prevalent. Because the book fills the need felt by all practitioners who have encountered mycotic infections and their clinical problems, it is a handy and useful book for the clinician.

One stated purpose of the book is clarification of mycotic nomenclature in the identification of pathogenic fungi.

Both the common and rarer mycoses are handled with masterly simplicity. From the various standpoints of symptomatology, differential diagnosis, prognosis, treatment, pathology, and immunology these infections are presented with clarity and thoroughness. Included among the cases discussed are such unusual conditions as South American Blastomycosis, and Histoplasmosis. Because geographical distribution of the mycoses is an important and essential factor in their identification care has been taken to point out the pathogenic fungi of certain regions. Discussion of laboratory methods for the identification and classification of fungi is complete and exhaustive.

Also included are discussions of the use of specific vaccines, both in diagnosis and in treatment of the mycoses. Justifiable is the emphasis placed on the use of these vaccines to determine the degree of sensitivity in the case of blastomycosis before instituting the iodide therapy, but still controversial is the issue among clinical dermatologists as to the use of vaccines in the treatment of the mycoses and their id reactions. While some clinicians attach little or no importance to the use of these the authors report favorable results in some cases. Prescriptions included are of proved effectiveness in treatment. The closing section is an illustrated study of the microscopic and macroscopic appearance of common contaminants.

Both the clinician and the mycologist will find use for this timely and worth while manual, for the number of these cases will continue to increase as the result of the geographic displacement of population coincidental to the war.

EARL R. COCKERELL.

THE EFFECT OF COX-TYPE VACCINE ON LOUSE-BORNE TYPHUS FEVER

An Account of 61 Cases of Naturally Occurring Typhus Fever in Patients Who Had Previously Received One or More Injections of Cox-type Vaccine

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INTRODUCTION

The attainment of successful immunization against epidemic louse-borne typhus fever has been one of the principal objectives in typhus research for many years. One of the early attempts to obtain a killed rickettsial vaccine was made by Da Rocha-Lima, who prepared a phenolized suspension of lice which had been allowed to feed on typhus patients (1). Weigl extended Da Rocha-Lima's observations by devising an ingenious technique for the inoculation of lice (2) and thereby made it possible to produce limited quantities of vaccine from louse intestines. The observers who administered Weigl's vaccine in Poland, China, and North Africa reported that the vaccine had a favorable effect in modifying the course of epidemic typhus (references cited in 3).

Although the work of Da Rocha-Lima and Weigl thus indicated the possibility of successful immunization against typhus fever with killed rickettsiae, the difficulties inherent in the preparation of louse vaccine rendered its utilization impractical on a large scale. Steps toward the solution of the problem of obtaining sufficiently large quantities of rickettsiae for the production of killed vaccines were made by Zinsser and Castaneda (4), who produced abundant rickettsiae from X-rayed rats, by Castaneda (5), who introduced the method for preparation of murine rickettsial vaccine from rodent lungs, and by Durand and Giroud (6), who prepared epidemic louse-borne rickettsial vaccine from mouse lungs. But a method even more practical and satisfactory was devised by Cox (7, 8, 9), who utilized the yolk sac membrane of developing chick embryos as a growth medium for rickettsiae of various types.

There were several other approaches to the question of typhus vaccination, such as the use of "attenuated" living rickettsiae. Since the entire subject of typhus vaccines has recently been reviewed in detail (3), this discussion will be restricted to a consideration of the effectiveness of Cox-type vaccine.

Following Cox's discovery, the observation was quickly made in several different laboratories that Cox-type vaccine was of value in protecting laboratory workers from the severe and frequently fatal form of typhus fever to which their laboratory work so often exposed them (10, 11, 12, 13). Repeated vaccination, to be sure, did not *prevent*

[1] Staff member, The Rockefeller Foundation, on leave.

infection among laboratory workers, but postvaccination cases were, for the most part, greatly modified. Indeed, a verified account of a single fatal case of proven typhus fever in a laboratory worker adequately vaccinated with Cox-type vaccine has not yet come to our attention. (The expression "adequate vaccination" will be discussed in a later part of this report).

In addition to the evidence from postvaccination laboratory infections, there is information bearing on the effects of Cox-type vaccine in humans who were given virulent challenge material. In Russia results were obtained which indicated that human volunteers who received three doses of Cox-type vaccine were completely protected against death from typhus following subcutaneous inoculation of a suspension of virulent guinea pig brain, but that larger quantities of vaccine were required to reduce the *morbidity* in that type of challenge experiment. The clinical picture of typhus after vaccination was definitely less severe than typhus in unvaccinated persons (14).

An account of the effect in humans of various types of vaccines, including the Cox type, has been published in Germany by Ding (15), whose report is of considerable interest though it omits much essential information, such as the number of persons involved, and the manner in which they were infected. Ding presented charts showing the average temperature curves of groups of persons who contracted typhus after vaccination. He included the average temperature curves of the unvaccinated control groups also. He stated that the date of infection and incubation period were accurately known in every instance. The reader can assume from the shortness of the incubation period in the control unvaccinated groups, that Ding performed a direct experiment using a very heavy challenge dose of rickettsiae. It can be inferred, moreover, from the percentages which are stated in the tables, that Ding probably used large numbers of subjects in each group. The mortality of his two control groups was 20 to 30 per cent. He concluded that Cox yolk sac vaccine, as well as the louse intestine and rodent lung vaccine (in each of which the concentration of rickettsiae probably was similar to that prepared according to Cox's method), protected against death from typhus but did not prevent the vaccinated persons from contracting mild infections. He stated that the fever was diminished in height and duration, and that complications were reduced in number and severity by the vaccines.

Although the evidence from laboratory infections and from the experiments reported by the Russians and the Germans is impressive, it is obvious that laboratory infections and human experiments of that type do not reproduce the conditions which prevail in populations suffering from an epidemic of louse-borne typhus fever where the mode of infection and the severity of exposure to typhus probably are quite different. Published evidence of the effectiveness of Cox-type vaccine under actual epidemic conditions is meager. The field studies of Cox vaccine which were attempted in Spain in 1941 (16) and in Bolivia in 1941–42 (17) did not yield definite results. A satisfactory field trial of Cox-type vaccine was, therefore, one of the principal objectives of the United States of America. Typhus Commission when it was formed in 1942 (18).

The field group of the U. S. A. Typhus Commission established its headquarters in Cairo, Egypt, in January 1943. With the cooperation of the Egyptian Ministry of Public Health, the Commission members had an opportunity to study the effect of ether extracted Cox-type vaccine in a large group of persons intimately exposed by their occupations to naturally acquired typhus fever during two severe epidemics. Although the data thus obtained are not derived from the ideal type of field trial with large control groups, nevertheless the observations are considered of sufficient interest to justify a report at this time.

The extent of the typhus epidemics in Egypt in 1943 and 1944.—Typhus fever has occurred every winter in Egypt for many years. Usually the outbreaks have varied from a few hundred to a few thousand cases, but in 1943 Egypt experienced the most severe epidemic in her recorded medical history, when approximately 40,000 cases were reported, with an overall mortality of 14 per cent. At the peak of the epidemic in April and May, 1943, more than 4,400 cases were admitted to the Cairo Fever Hospital at Abassia. In 1944 the incidence of cases in Cairo itself was approximately half that in 1943, with no diminution in mortality rate.

Subjects of the vaccine study.—With such an exceptionally large number of typhus cases being admitted to the Cairo Fever Hospital it is obvious that the hospital employees were unusually exposed to the infection in the course of their duties in handling the typhus cases. Furthermore, many of these employees lived in the areas of the city

where typhus attack rates were high and were at risk there as well as in the hospital.

Before 1943 typhus vaccine had not been administered to this group. In the 18 month period immediately preceding this study there were 25 cases and eight deaths from typhus among the hospital employees. As the epidemic spread in 1943 many new employees were added to the hospital staff to handle the large increase in typhus cases. In 1943 there was only one hospital in the Cairo area which admitted typhus patients (called the Cairo Fever Hospital at Abassia). In 1944 a second hospital for typhus was established (called the Embaba Hospital).

The employees of both hospitals were included in the vaccination program which was undertaken by the U. S. A. Typhus Commission in cooperation with the Egyptian officials.

Plan of the vaccination program.—The U. S. A. Typhus Commission members vaccinated all those employees of the Fever Hospitals who appeared at the designated vaccination post each week. Since some of the employees did not desire to be vaccinated there was a group of persons who did not receive any vaccine. This unvaccinated group was considerably smaller than had been anticipated, probably because most of the employees quickly became convinced that their comrades who acquired typhus after vaccination were much less severely ill than unvaccinated typhus patients.

Immunization records of each employee were kept by the Commission. Most of the employees who contracted febrile illnesses, whether vaccinated or not, were seen during their hospitalization by one or more of the Commission members. Owing to difficulties outside the scope of our discussion, the clinical and laboratory information which was obtained from many of the postvaccination typhus cases in 1943 was not as complete as we had desired, but in 1944 it was possible to admit several of the cases to the Commission ward for thorough clinical and laboratory studies and to follow the employees in the general wards of both Abassia and Embaba more closely, particularly from the clinical point of view. The records of the 1943 cases have been reviewed and those with inadequate information have been excluded from consideration in this report. The remaining 1943 records have been combined with the 1944 records to form the group of 61 postvaccination cases which are now presented. The cases seen in 1943 are designated by the letter G before their hospital numbers. Among the cases seen in 1944, the letter E indicates that the case occurred in an Embaba Hospital employee; hospital numbers without a letter indicate cases at the Abassia Hospital.

Schedule of vaccination.—Ether extracted Cox-type yolk sac vaccine equivalent in concentration to 10 per cent yolk sac suspensions of rickettsia of epidemic typhus (19) was used throughout. This vaccine met the potency requirements of the National Institute of Health U. S. Public Health Service. The aim was to give three subcutaneous injections of 1 cc. each at weekly intervals and to give stimulating doses of 1 cc. at intervals of 4 to 6 months. Irregularity in attendance of the employees created aberrations in this schedule in many instances.

TABLE I

Typhus fever in Cairo Fever Hospital employees

TOTAL NUMBER OF HOSPITAL EMPLOYEES	NUMBER WHO RECEIVED 1 OR MORE DOSES OF VACCINE	CASES OF TYPHUS AMONG THE HOSPITAL EMPLOYEES*	
		No vaccine	After 1 or more doses of vaccine
Jan. to July 1943			
Abassia: 879	743	8	33
Nov. 1943 to Sept. 1944			
Abassia: 830	799	1	14
Embaba: 517	460	1	14

* These do not include all cases among the 879 employees diagnosed as typhus by the Fever Hospital physicians. Seven additional cases were reported, which are excluded here because of inadequate information in their records.

Local reactions to vaccination (redness and transient tenderness) were infrequent and generalized reactions were not observed.

In 1943 vaccination was begun in February and continued until July. In the second season vaccination was carried out between November, 1943, and September, 1944. Table I shows the number of employees, the number vaccinated with one or more doses, and the cases of typhus among the employees in both seasons.

Diagnosis of typhus fever after vaccination.—Despite the mild course which distinguished many of the cases of postvaccination typhus, it was usually possible to recognize the disease on clinical grounds alone. Results of laboratory tests in nearly every case confirmed the clinical diagnosis.

In some instances, however, the diagnosis of typhus was made *only* with the aid of laboratory evidence, i.e., rise in titer of Weil-Felix and complement fixation tests during the illness or in early convalescence. The serologic criteria for the diagnosis of postvaccination typhus have been worked out by Zarafonetis (20) who has reported his results in other communications. In brief, he found that nontyphus febrile illnesses do not evoke high complement fixation titers in the sera of persons who have previously received multiple doses of Cox-type vaccine, including various numbers of stimulating doses. On the other hand, he found that known typhus infections regularly do stimulate production of high complement fixation titers in the sera of patients who have been vaccinated previously. Stimulating doses do not produce rises in titer of the magnitude found after known typhus infection in previously vaccinated persons. The results of Weil-Felix tests were usually similar to results of the complement fixation tests.

Since the serologic criteria as established in Zarafonetis' work were based on studies of 100 known nontyphus febrile illnesses and sixteen definite cases of postvaccination typhus,[2] we have applied his results with confidence to the interpretation of the changes in titers observed in vaccinated patients in the present study, particularly when clinical evidence alone was inadequate for the basis of a diagnosis of typhus, either because of insufficient observation of the patient or because of the unusually mild course of the infection.

Strains of rickettsiae were isolated from the blood of a few of the cases by Colonel Plotz in 1943 and by the authors in 1944. Although our studies are not yet complete, we have preliminary evidence which indicates that the blood of several cases of postvaccination typhus was infectious for human lice. These results will be described in detail when current studies are completed.

Estimation of severity of illness.—To facilitate the analysis of cases an arbitrary classification of clinical severity was adopted. After discharge from the hospital each patient was classified on the basis of his clinical course. The principal factors which influenced the estimation of severity were the intensity of subjective symptoms (headache, generalized bodily aches and pains, tinnitus, deafness); the degree of prostration; the extent of central nervous system involvement (mental dullness, stupor, coma, incontinence of urine and feces, abnormal neurological signs); the severity of cardiovascular system involvement (hypotension, tachycardia, peripheral vascular failure, myocardial damage); and finally, the occurrence of urinary retention, oliguria, nitrogen retention, bronchopneumonia, otitis media, parotitis, furunculosis, and gangrene. With these factors in mind the following classification was made:

"A": Cases so mild that a definite diagnosis of typhus on clinical evidence alone was not possible, the final diagnosis being made only with the aid of positive laboratory data.

"B": Cases with minimal symptoms and signs, yet definitely diagnosed as typhus on clinical evidence.

"C": Cases of moderate severity, showing slight prostration, central nervous system involvement, cardiovascular changes, or mild complications.

"D": Severe typhus fever cases, with marked prostration, central nervous system involvement, cardiovascular changes, or serious complications.

"E": Cases so severely ill that a fatal outcome was expected at some point in the clinical course.

"F": Fatal cases.

The clinical classification of severity was devised as a method for tabulating the severity of the individual case. In order to indicate the severity of disease in a whole group, arbitrary numerical values, increasing with each increase in severity, were assigned to the clinical classifications. When applied to each case in a series, these figures have been added and averaged to arrive at an index for comparison of different groups. The following values were assigned to each classification: "A" cases, 10; "B", 20; "C", 30; "D", 40; "E", 50; and "F", 60.

Definitions and assumptions.—The term "one dose of vaccine" is defined as a single subcutaneous injection of 1 cc.; "two doses of vaccine" as two injections of 1 cc. each, etc.

The interval of 10 days is accepted as the usual incubation period of louse-borne typhus fever. The "probable date of infection" of each case can therefore be said to have occurred 10 days before the onset of the disease.

It is assumed that a period of 10 to 12 days must

[2] By definite cases of postvaccination typhus we mean those patients from whom strains were isolated or whose clinical course was unquestionably typhus.

elapse after a dose of typhus vaccine before any immunity can be considered to have developed as a result of that injection of typhus vaccine.

On the basis of these assumptions, we have arranged our postvaccination patients in the following groups in order to facilitate an evaluation of their severity in relation to the amount of vaccine and the interval between vaccination and onset of illness:

Group 1: Onset of typhus 21 days or more after the 3rd dose of vaccine.

Group 2: Onset of typhus 21 days or more after the 2nd dose of vaccine.

Group 3: Onset of typhus 21 days or more after the 1st dose of vaccine.

Group 4: Onset of typhus less than 12 days after the 1st dose of vaccine.

Several of the patients in Group 1, 2, and 3 received additional doses of vaccine at some time during the 21 day period preceding the onset of their illness. These additional doses have been disregarded in the arrangement of cases according to the above classification, but the number of extra doses in relation to onset of illness is stated for each patient in the column marked "Notes" in Table V.

Background for the evaluation of the results of vaccination.—As already noted, there was no strictly comparable group of unvaccinated control subjects in this clinical study. It was not considered justifiable to withhold vaccination from any of the hospital employees who were so greatly at risk under these conditions. However, data are available from the different groups which supply information concerning the severity of typhus fever among unvaccinated Egyptians.

The first unvaccinated group is composed of ten hospital employees (seven females; three males; ages 18 to 45 years) who refused vaccination and contracted typhus in the course of their duties. They were cared for in the general wards of the Fever Hospital. The second unvaccinated group is composed of 44 male Egyptians, ages 18 to 48 years, whose clinical course was thoroughly studied in the Commission ward where they received no specific treatment except adequate fluids, good nursing, and energetic measures to combat those complications which arose in the course of their illness.

These two groups are referred to as the "cordon cases" and the "Commission ward cases", respectively. In offering them for comparison with the vaccinated cases we are fully aware of the objections which can be raised to this procedure. On the other hand, the course of epidemic louse-borne typhus in unvaccinated, untreated Egyptians as seen in the Cairo Fever Hospital was severe and entirely typical of the disease as it has occurred in epidemics in other countries. The possibility exists that some of these patients may have had typhus in infancy and therefore would be expected to suffer only mild forms of the disease in second attacks as adults. That this consideration may be dismissed as of minor importance in Cairo in 1943 and 1944 is shown by the high mortality of the disease in the age groups (16–48) which are most pertinent to this study (see Table II).

Course of typhus fever in unvaccinated Egyptians.—The severity of illness and duration of fever in relation to age for the 44 *unvaccinated* Commission ward cases are shown in Table III. The cases were classed as follows: 1 "B", 12 "C", 18 "D", 5 "E", and 8 "F". In spite of hospitalization during the first week of illness and good nursing care, the mortality was 18 per cent, and the average duration of fever was 18½ days for the nonfatal cases.

TABLE II

Typhus fever in the Cairo Fever Hospital (Abassia) between January 1, 1943 and September 1, 1944: Age-specific case fatality rates

AGES	MALES			FEMALES		
	Cases	Deaths	Mortality	Cases	Deaths	Mortality
			per cent			*per cent*
16–20	1247	120	9.6	689	60	8.7
21–25	1363	208	15.2	586	61	10.4
26–30	988	252	25.5	540	74	13.7
31–35	598	184	30.8	377	71	18.8
36–40	422	142	33.6	232	59	25.4
41–48	264	124	47.0	135	44	32.6

The course of typhus in the ten unvaccinated hospital employees treated in the general hospital wards is shown in Table IV. The average duration of fever was 15½ days in the nonfatal cases. There were seven females, average age 26 years, and three males, average age 27 years.

Course of typhus fever in vaccinated Egyptians.—Group 1 (three doses of vaccine at least 21 days prior to onset).—Clinical notes from the records of five patients who were thoroughly studied on the Commission ward are presented to illustrate some of the features of postvaccination typhus (see Table V).

TABLE III

Forty-four unvaccinated male Egyptian typhus fever patients admitted to the commission ward in the first week of illness

HOSPITAL NO.	AGE	DURATION OF FEVER*	CLINICAL CLASSIFICATION OF SEVERITY†
	years	*days*	
7654	18	21	D
1197	18	18	E
5587	19	38	E
558	20	16	C
5043	20	17	C
6243	20	16	C
16929	20	19	D
530	21	14	C
3195	21	16	C
4114	21	16	D
5754	21	25	D
19094	21	35	D
6568	21		F
18347	22	10	C
375	22	14	D
1525	22	17	D
2780	22	19	D
3799	22	27	D
2422	23	16	D
2735	23	15	D
6339	24	16	C
1896	25	17	C
3919	25	15	C
5508	25	27	D
8119	25	19	D
5808	25	18	E
1690	26	12	C
6146	26		F
18989	27	18	B
1495	27	14	D
12412	28	15	D
885	29	14	D
15006	30	18	D
5133	30		F
7250	30		F
5708	32	18	C
17035	32	13	D
1902	35	27	E
5585	35	16	E
2621	35		F
3307	36		F
7464	43		F
5769	46		F
835	47	14	C

* Rectal temperatures were taken every 4 hours. A value above 37.5°C. once or more during the 24 hour period was called a day of fever.

† The letters are explained in the text.

№ 1680. 3 doses of vaccine, the last dose 39 days before onset. Male, 45 years. This patient had a severe chill the first day combined with frontal headache and, later, joint pains. Rash was moderate. His mild course was marked latterly by the development of bilateral costovertebral pain and microscopic hematuria. After 12 days of moderate elevation of temperature he continued to maintain a low grade fever until the 21st day. His temperature record is given in Fig. 1. (The importance of his hematuria in relation to typhus is not clear; bilharzia infection in Egypt is widespread. The rôle of the kidney in typhus is discussed elsewhere (21).) Clinical classification of severity: "B."

№ 2653. 5 doses of vaccine, the last dose 117 days before onset. Male, 26 years. Typhus Commission field worker. He had rather severe headache and malaise. The rash was fleeting but definite. With the history and the rash no difficulty was experienced in making the diagnosis, though the course was mild. Fever lasted 9 days. Clinical classification of severity: "B."

TABLE IV

Ten unvaccinated Fever Hospital employees who contracted typhus (cordon cases)

HOSPITAL NO.	SEX	AGE	DURATION OF FEVER*	CLINICAL CLASSIFICATION OF SEVERITY†
		years	*days*	
G 5204	M	18	13	C
G 2919	M	28	14	C
G 3709	M	34		F
G 169	F	18	14	C
G 5239	F	19	20	D
2798	F	20	18	D
E 5287	F	25		F
G 1111	F	25	13	C
G 1060	F	30	15	C
G 2994	F	45		F

* Temperatures were taken orally, at least twice daily, morning and night. Oral temperatures above 37.0°C. are counted as indicating a day of fever.

† The letters are explained in the text.

№ 2657. 3 doses of vaccine, the last dose 87 days before onset. Male, 45 years. He appeared to be at least 55 years old. He was never especially ill during his course. He developed a tremor of his limbs, almost Parkinsonian in character. This disappeared during convalescence. Fever lasted 12 days. Clinical classification of severity: "B."

№ 2799. 3 doses of vaccine, the last dose 103 days before onset. Male, 18 years. This was a problem in diagnosis. Moderately ill; said he felt "weak," but had no specific complaint. There was nothing to be found but a very few fleeting macules requiring careful

search. There was no conjunctival injection. Fever lasted 10 days. Clinical classification of severity: "B."

#2805. 5 doses of vaccine, the last dose 79 days before onset. An obese male, age 33 years. He complained of generalized body pains, especially in the knees, and said he felt "feverish." He had severe headache. There was some tinnitus and slight deafness. His course was mild. Fever lasted 8 days. Clinical classification of severity: "B."

classed as follows: 4 "B", 3 "C". Four were females, average age 19 years. Three cases were males, average age 28 years.

Group 3 (one dose of vaccine at least 21 days prior to onset): The data from the eleven patients in Group 3 are shown in Table V. The average duration of fever was 12.7 days. The cases were classed as follows: 2 "B", 8 "C", and 1 "D"

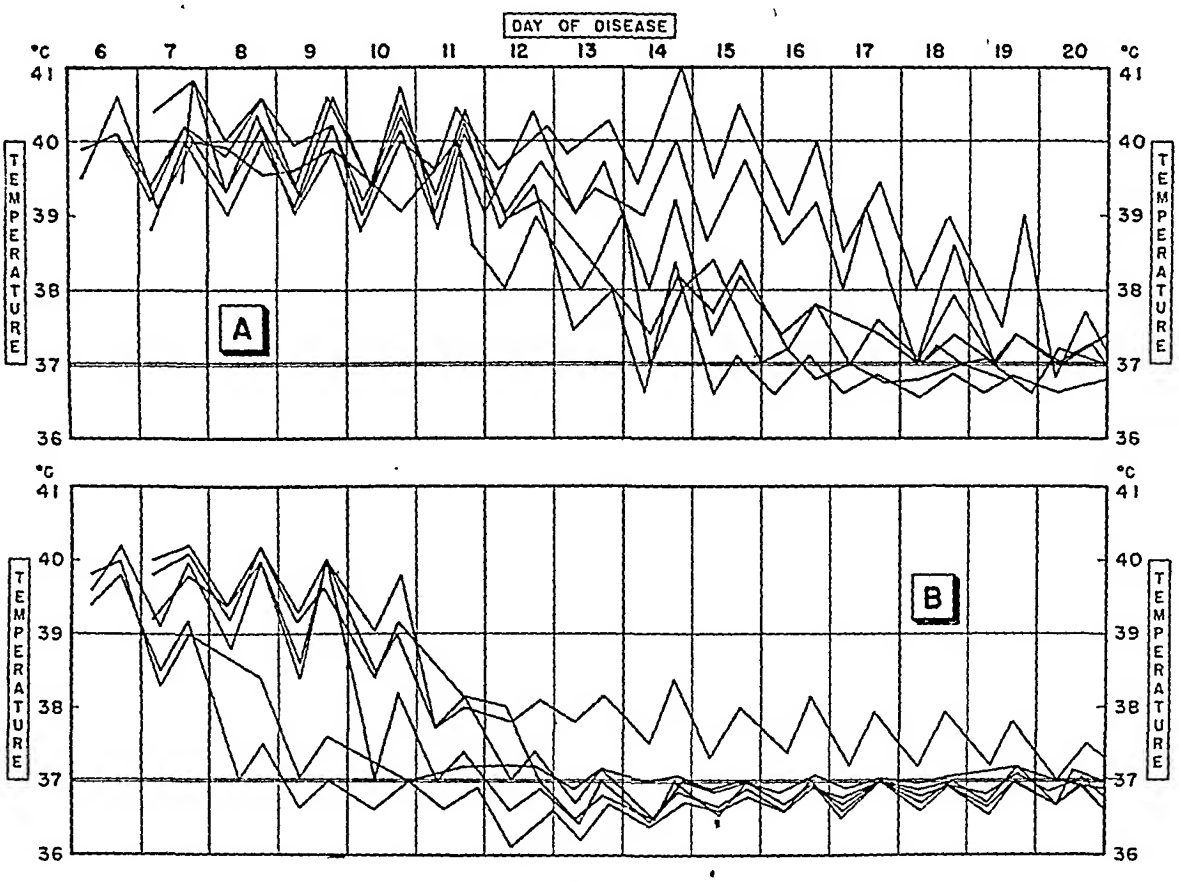

FIG. 1. A: Superimposed temperature records of six non-fatal, unvaccinated control cases selected at random B: Superimposed temperature records of six vaccinated cases (three or more injections) studied on the ward.

The data from the remaining 21 patients in Group 1 who were observed in the cordon are shown in Table V.

The average duration of fever for all the patients in Group 1 was 10½ days. The cases were classed as follows: 1 "A", 20 "B", 5 "C", none in "D", "E", or "F". Fifteen of the cases were females, average age 22.7 years; eleven cases were males, average age 31.8 years.

Group 2 (two doses of vaccine at least 21 days prior to onset): The data from the seven patients in Group 2 are shown in Table V. The average duration of fever was 12 days. The cases were

Five were females, average age 21 years. Six cases were males, average age 34 years.

Group 4 (onset of typhus less than 12 days after first dose of vaccine): The data from the seventeen patients in Group 4 are shown in Table V. (#2002 was a Commission ward case, the others were observed in the cordon). The average duration of fever of the nonfatal cases was 15 days. The cases were classed as follows: 4 "B", 8 "C", 4 "D", and 1 "F". There were twelve females, average age 23.6 and five males, average age 27.8 years.

TABLE V

Data pertaining to 61 patients who contracted typhus fever after varying amounts of Cox type vaccine

Vaccine group*	Hospital number	Sex	Age	Number of doses of vaccine	Interval between last dose and onset of typhus	Duration of fever	Clinical classification of severity*	Rash†	Maximum titer of serologic tests‡			Notes
									Day of illness when sample obtained	Complement fixation (epidemic antigen)	Weil-Felix (Proteus OX19)	
					days	*days*						
1	2799	M	18	3	103	10	B	+	12	1/1024	1/80	Difficult clinical diagnosis
	2653	M	26	5	117	9	B	++	19	1/512	1/160	Worked during first few days of illness
	G 9197	M	27	3	25	8	B	±	33	1/320	1/160	
	E 256	M	28	3	50	8	A	0	19	1/512	1/40 C	Difficult clinical diagnosis
	E 3612	M	28	4	87	10	B	++	9	1/8192	1/2560	
	4930	M	29	4	26	11	B	++	12	1/160	1/640	
	2805	M	33	5	79	8	B	+	13	1/512	1/80	
	1886	M	35	3	325	10	B	+	35	1/1024	1/320	Fever Hospital diagnosis "influenza"
	1988	M	36	3	200	15	C	+	19	1/2048	1/2560	
	1680	M	45	3	39	21	B	++	8	1/1024	1/160	Back pain and hematuria; several days low fever
	2657	M	45	3	87	12	B	++	16	1/1024	1/160	Prominent tremor, general. Never seriously ill
	G 5601	F	16	3	21	7	B	+	9	1/1280	1/160	
	E 589	F	16	3	71	10	B	±	37	1/1024	1/640	Worked during first few days of illness
	G 8989	F	17	3	55	9	B	+	10	1/320	1/1280	"Washed out. Not toxic"
	G 11707	F	17	3	38	11	C	++	8		1/250[b]	"Somewhat delirious"
	G 3292	F	17	3	25	5	B	±	13	1/640	1/640	
	E 2863	F	18	3	138	11	B	+	19	1/4096	1/160	
	21434	F	18	3	161	7	B	+	11	1/8192	1/160	Difficult clinical diagnosis
	G 9032	F	18	3	23	13	C	+	10	1/320	1/80	Slightly toxic
	E 270	F	19	3	52	7	B	0	12	1/4096	1/1280	Worked during first few days of illness
	E 265	F	20	3	48	10	B	0	16	1/512	1/640	Femoral phlebitis
	1722	F	22	5	69	13	B	++	12	1/4096	1/320	
	G 12835	F	30	3	60	11	B	+	12	1/320	1/40	
	6056	F	35	4	56	11	B	++	30	1/2048	1/1280	
	G 5392	F	35	3	21	8	C	+	11	1/320	1/640	Definitely toxic. Another dose of vaccine 13 days before onset
	Mag.[c]	F	43	4	146	13	C	+++	33	1/256	1/160	Ambulatory first week

2	4133	M	23	2	22	9	B	++	10	1/512	1/160	Not very sick. Another dose of vaccine 15 days before onset
	G 12000	M	30	2	62	14	C	+	16	1/640	1/40 P	Moderately toxic
	G 8583	M	30	2	78	9	C	++	10	1/1280	1/2560	Definitely toxic
	G 3022	F	16	2	30	9	B	++	15	1/320	1/40	Another dose of vaccine 19 days before onset
	E 198	F	17	2	42	14	C	+	20	1/256	1/640	Slight stupor
	G 3023	F	20	2	30	14	B	++	12	1/320	1/640	Another dose of vaccine 19 days before onset
	G 5289	F	20	2	36	14	B	+	17	1/640	1/160	Two more doses vaccine 19 and 3 days before onset
3	G 9997	M	25	1	23	17	D	++	11	1/160	1/1280	
	G 8619	M	30	1	68	12	B	++	15	1/640	1/640	
	G 5764	M	31	1	21	9	C	++	9	1/640	1/1280	Slightly toxic. Two more doses vaccine 11 and 1 days before onset
	G 9145	M	35	1	29	13	C	++	11	1/320	1/60 P	Toxic. Two more doses vaccine 15 and 8 days before onset
	G 3928	M	40	1	45	15	C	+	14	1/40	1/192	NPN 47 mg. %
	G 3303	M	43	1	38	12	C	±	16	1/320	1/40	
	E 951	F	18	1	64	17	C	++	20	1/2560	1/1024	Some deafness. One more dose vaccine 2 days before onset
	E 3528	F	18	1	42	14	C	+	11		1/320	Slight deafness
	G 9191	F	20	1	36	10	C	0	13	1/1280	1/80 P	Two more doses vaccine 20 and 13 days before onset
	E 3942	F	25	1	64	9	B	±	18	1/320	1/2560	Six months pregnant. No miscarriage
	G 6572	F	25	1	46	12	C	+++	10	1/320	1/160	
4	2002	M	22	1	8	29	D	+++	33	1/1024	1/640	Broncho-pneumonia. Normal temperature 17th day
	G 5274	M	26	1	3	13	B	+	42	1/40	1/320[c]	
	G 5143	M	27	1	11	14	C	++	11	1/160	1/640	Another dose of vaccine on the day of onset
	G 9897	M	29	1	1	18	C	+++	17	0	1/640	
	G 5146	M	35	1	3		F	++	8	1/6	1/80	Died 17th day. Strain isolated
	G 3712	F	16	1	3	14	C	++	11		1/250	Slight deafness
	G 6296	F	16	1	3	17	C	++	19	1/1280	1/640	
	E 3199	F	19	1	6	14	D	++	30	1/256	1/1280	
	G 7104	F	20	1	3	11	B	0	14	1/640	1/160	Difficult clinical diagnosis
	E 5066	F	20	1	2	15	C	++	8	1/8	1/320	
	G 5675	F	20	1	10	13	C	+++	10	1/320	1/160[d]	Another dose of vaccine on the day of onset
	G 6394	F	25	1	13	11	C	++	11	1/1280	0	Another dose of vaccine 4 days after onset
	G 5224	F	25	1	9	12	C	++	11	1/160	1/5120	

TABLE V—*Continued*

VACCINE GROUP*	HOSPITAL NUMBER	SEX	AGE	NUMBER OF DOSES OF VACCINE	INTERVAL BETWEEN LAST DOSE AND ONSET OF TYPHUS	DURATION OF FEVER	CLINICAL CLASSIFICATION OF SEVERITY*	RASH†	MAXIMUM TITER OF SEROLOGIC TESTS[a]			NOTES
									Day of illness when sample obtained	Complement fixation (epidemic antigen)	Weil-Felix (Proteus OX19)	
					days	*days*						
4 (cont.)	E 1151	F	28	1	5	14	D	±	16	1/2040	1/5120	Delirium
	E 1463	F	30	1	3	13	B	++	13	1/256	1/1280	Five months pregnant. No miscarriage
	G 5212	F	30	1	7	12	D	+	67	1/320	1/1280	
	G 5655	F	35	1	9	11	B	+	12	1/160	1/640	

* Group 1—three doses of vaccine at least 21 days prior to onset. Group 2—two doses of vaccine at least 21 days prior to onset. Group 3—one dose of vaccine at least 21 days prior to onset. Group 4—onset of typhus less than 12 days after first dose of vaccine.

† Description of rash: None, 0; Questionable, ±; Slight, +; Moderate, ++; Profuse, +++.

[a] Tests on cases prefixed by the letter G were performed by Colonel Harry Plotz and Captain B. Bennett. The technique of the tests was similar to that employed for the other cases (26). The values stated in the table were selected from the series of samples for each patient to demonstrate maximum titers insofar as our data permitted. The letters P and C after Weil-Felix titers indicate complete and partial agglutination.

[b] End point not determined. This result obtained by the Fever Hospital laboratory where 1/250 is the highest dilution tested.

[c] The Weil-Felix titer was obtained on the 11th day of illness.

[d] The Weil-Felix titer was obtained on the 4th day of illness.

[e] Mag. refers to a nurse who was not hospitalized in Abassia.

Comparison of the fever charts of vaccinated and unvaccinated cases.—Fig. 1 shows the temperature curves of cases studied in the Commission ward. The records of six unvaccinated patients were selected at random from the 44 male cases. Their temperature curves are shown in Part A of Fig. 1. The records of six male patients who had received three or more injections of Cox vaccine are shown in Part B.

ANALYSIS OF THE DATA

The details in the foregoing tables are summarized in Table VI which is arranged to show the clinical severity and duration of fever in relation to the amount of vaccine received. The 44 unvaccinated Commission ward cases (all males) and the ten unvaccinated cordon cases (three males, seven females) are considered together as one group. The postvaccination male and female hospital employees are likewise considered together in this table. The average age of each group is indicated.

It is apparent that the patients who received three or more doses of vaccine 21 days or more before the onset of typhus had the mildest course and the shortest febrile period. As the amount of vaccine decreased, the clinical severity and the duration of fever increased. The only fatal case in any of the vaccine groups occurred in Group 4 (patients who were in the incubation period of typhus when vaccination was first begun). For convenience, Group 1, 2, and 3 are combined into a single group at the bottom of the table. There is a striking contrast between the 54 unvaccinated cases and the 44 cases in Vaccine Group 1, 2, and 3 as regards clinical severity and duration of fever. Furthermore, there were eleven fatal cases in the unvaccinated; none in Group 1, 2, and 3. The numerous differences which were noted between the unvaccinated and the vaccinated cases, such as the incidence and severity of complications, the degree of prostration, the occurrence of nitrogen retention, and so forth, are summarized in the clinical classification of severity which is based on the combined evidence from each patient's record.

To what factors may these differences between the unvaccinated and the vaccinated patients be attributed? The figures for all of the typhus cases admitted to the Cairo Fever Hospital (see Table II) indicate a rise in mortality with increasing age, the usual relationship observed in all louse-borne typhus epidemics. Further, it is clear that females had a lower mortality than males in the same age groups, a phenomenon which has occasionally been noted in other countries (22). In view of these facts, it is necessary to determine whether the mildness of postvaccination typhus in this group of hospital employees can be attributed to differences in the sex and age distribution of the patients. To facilitate an analysis of these factors the cases have been arranged according to sex and age in Table VII, VIII, IX, and X.

Severity of postvaccination typhus in male patients.—The data from the male patients are presented in Table VII and IX. The ages of the postvaccination male patients were greater than those of the unvaccinated male patients. It is clear that there was a very definite difference in clinical severity between the unvaccinated males and the Group 1 males. The severity of Group 1, 2, and 3 combined, likewise shows a difference from the unvaccinated cases. The Group 4 cases are discussed in a later section.

Can these differences be attributed to chance? One approach to this question is a calculation of the coefficient of correlation between the amount of vaccine and the clinical severity of the cases. To reduce complicating factors to a minimum, the calculation is restricted to the male hospital employees only, since these patients form the most nearly homogeneous group in the study. The group is composed of twenty patients who received one or more doses of vaccine 21 or more days before the onset of illness, and three patients who contracted typhus without previous vaccination. The patients who were first vaccinated during the incubation period of typhus are not included in the calculation. Doses of vaccine given less than 21 days before onset are not counted. The mean amount of vaccine for the 23 patients was 2.22 cc., standard deviation $\pm$ 1.51. The mean severity was 26, the standard deviation $\pm$ 9.86. The coefficient of correlation r between the amount of vaccine and the clinical severity was -0.644, which is more than three times the standard error of the number. Reference to Table VI in Fisher and Yates (23) shows that with such a value of r it is highly unlikely that the correlation observed between the amount of vaccine and the clinical severity of postvaccination typhus was due to chance.

An obvious factor which might account for the observations in this group of 23 patients would be an age distribution such that those cases which had less vaccine were the older patients who might reasonably be expected to undergo a more severe clinical course. Inspection of Table IX suggests

TABLE VI

Summary of data from male and female typhus patients, including commission ward and cordon cases

VACCINE GROUP*	TOTAL NUMBER IN GROUP	AVERAGE AGE	AVERAGE DURATION OF FEVER	NUMBER OF PATIENTS IN EACH CLINICAL CLASSIFICATION OF SEVERITY						INDEX OF SEVERITY †
				A	B	C	D	E	F	
		years	*days*							
Group 1	26	26.6	10.5	1	20	5	0	0	0	22
Group 2	7	22.3	12.5	0	4	3	0	0	0	24
Group 3	11	28.2	12.5	0	2	8	1	0	0	30
Group 4	17	24.9	15	0	4	8	4	0	1	32
No vaccine	54	26.4	18	0	1	17	20	5	11	41
Total, Group 1, 2, and 3 combined	44	26.4	11	1	26	16	1	0	0	23

* Group 1—three doses of vaccine at least 21 days prior to onset. Group 2—two doses of vaccine at least 21 days prior to onset. Group 3—one dose of vaccine at least 21 days prior to onset. Group 4—onset of typhus *less than 12 days after first dose of vaccine.*

† Index of severity is defined in the text.

TABLE VII

Comparison of unvaccinated and vaccinated Egyptian male typhus patients

VACCINE GROUP*†	TOTAL NUMBER IN GROUP	AVERAGE AGE	AVERAGE DURATION OF FEVER	NUMBER OF PATIENTS IN EACH CLINICAL CLASSIFICATION OF SEVERITY						INDEX OF SEVERITY†
				A	B	C	D	E	F	
		years	*days*							
Group 1	11	31.8	11.1	1	9	1	0	0	0	20
Group 2	3	27.7	10.7	0	1	2	0	0	0	27
Group 3	6	34	13.0	0	1	4	1	0	0	30
Group 4	5	27.8	18.5	0	1	2	1	0	1	34
No vaccine (commission ward and cordon cases combined)	47	26.4	18.1	0	1	14	18	5	9	42
Total, group 1, 2, and 3 combined	20	31.9	11.6	1	11	7	1	0	0	24

*† See footnotes Table VI.

TABLE VIII

Comparison of unvaccinated and vaccinated Egyptian female typhus patients (hospital employees)

VACCINE GROUP*	TOTAL NUMBER IN GROUP	AVERAGE AGE	AVERAGE DURATION OF FEVER	NUMBER OF PATIENTS IN EACH CLINICAL CLASSIFICATION OF SEVERITY						INDEX OF SEVERITY†
				A	B	C	D	E	F	
		years	*days*							
Group 1	15	22.7	9.7	0	11	4	0	0	0	23
Group 2	4	18.3	12.8	0	3	1	0	0	0	23
Group 3	5	21.2	12.4	0	1	4	0	0	0	28
Group 4	12	23.6	13.1	0	3	6	3	0	0	30
No vaccine‡	5	21.4	16.3	0	0	2	2	0	1	40
Total, group 1, 2, and 3 combined	24	21.7	10.8	0	15	9	0	0	0	22

*† See footnotes Table VI.

‡ This group does not include two unvaccinated hospital employees, ages 30 and 45, who were C and F respectively, in order to make the age distribution more nearly comparable to that of the vaccinated groups.

that this was not the case and calculation of the appropriate coefficients of correlation confirms that impression. The age distribution of the 23 male hospital employees, therefore, does not explain the milder course of those who received more vaccine.

Severity of postvaccination typhus in female patients.—The data from the female patients are shown in Table VIII and X. There was a preponderance of cases in the age group 16–20. There was no group of well-studied, unvaccinated female Commission ward typhus patients for comparison with the postvaccination cases, and only seven unvaccinated female cordon cases. The same relationship was apparent between clinical severity and amount of vaccine for female patients as for males. The correlation coefficient for female patients, obtained in the manner described in the preceding section, was −0.635. This value indi-

TABLE IX

Data from male Egyptian typhus cases arranged according to age and amount of vaccine

AGES	AMOUNT OF VACCINE																			
	Unvaccinated cases				Group 1 cases*				Group 2 cases				Group 3 cases				Total, group 1, 2, and 3 combined			
	Number of patients	Duration of fever	Index of severity†	Mortality	Number of patients	Duration of fever	Index of severity	Mortality	Number of patients	Duration of fever	Index of severity	Mortality	Number of patients	Duration of fever	Index of severity	Mortality	Number of patients	Duration of fever	Index of severity	Mortality
		days		*per cent*		*days*		*per cent*		*days*		*per cent*		*days*		*per cent*		*days*		*per cent*
16–20	8	19.7	38	0	1	10.0	20	0									1	10.0	20	0
21–25	19	18.7	38	5.3					1	9.0	20	0	1	17	40	0	2	13.0	30	0
26–30	10	15.0	42	30.0	5	9.2	18	0	2	11.5	30	0	1	12	20	0	8	10.1	21	0
31–35	6	18.5	48	33.3	2	9.0	20	0					2	11	30	0	4	10.0	25	0
36–40	1	-	60	100	1	15.0	30	0					1	15	30	0	2	15.0	30	0
41–48	3	14.0	50	66.7	2	16.5	20	0					1	12	30	0	3	15.0	23	0
Total	47	18.1	42	19.0	11	11.1	20	0	3	10.7	27	0	6	13	30	0	20	11.6	24	0

*† See footnotes, Table VI.

TABLE X

Data from female Egyptian typhus cases arranged according to age and amount of vaccine

AGES	AMOUNT OF VACCINE																			
	Unvaccinated cases				Group 1 cases*				Group 2 cases				Group 3 cases				Total, group 1, 2, and 3 combined			
	Number of patients	Duration of fever	Index of severity†	Mortality	Number of patients	Duration of fever	Index of severity	Mortality	Number of patients	Duration of fever	Index of severity	Mortality	Number of patients	Duration of fever	Index of severity	Mortality	Number of patients	Duration of fever	Index of severity	Mortality
		days		*per cent*		*days*		*per cent*		*days*		*per cent*		*days*		*per cent*		*days*		*per cent*
16–20	3	17.3	36.7	0	10	9.0	22	0	4	12.8	23	0	3	13.7	30	0	17	10.7	24	0
21–25	2	13.0	45	50	1	13.0	20	0					2	10.5	25	0	3	11.3	23	0
26–30	1	15.0	30	0	1	11.0	20	0									1	11.0	20	0
31–35					2	9.5	25	0									2	9.5	25	0
36–40																				
41–48	1		60	100	1	13.0	30	0									1	13.0	30	0
Total.....	7	16.0	48	28.6	15	9.7	23	0	4	12.8	23	0	5	12.4	28	0	24	10.8	22	0

*† See footnotes, Table VI.

cates that the observed relationship between clinical severity and amount of vaccine would occur by chance only once in more than a thousand samples of this size. There was no unusual distribution of ages in the groups which received different amounts of vaccine, which might explain the differences in severity.

Effect of vaccination on mortality.—The age-specific case fatality rates in Table II, representing the entire population of typhus cases admitted to the Cairo Fever Hospital in 1943 and 1944 in the age group 16–48, have been used to calculate the expected number of deaths among a group of 61 persons with the same age and sex distribution as the postvaccination cases in this study. The theoretical number of deaths would be eleven. Actually, one death occurred in the vaccinated group (a patient who received one dose of vaccine only 3 days before the onset of illness). A comparison has been made to determine the statistical significance of this difference.

	SURVIVED	DIED	TOTAL
Vaccinated cases	60	1	61
Theoretical cases, death rates from Table II	50	11	61
	110	12	122

Chi square for this table is 9.23 and the corresponding value of P lies between 0.01 and 0.001. This difference in mortality between the vaccinated patients and an equal number of Egyptians of the same sex and ages would be expected to occur by chance only once in more than a hundred samples of this size.

If we consider as "vaccinated" only those patients in Table V who had one or more doses of vaccine *5 days* or more before the onset of typhus, in comparison with a similar group of the same size (mortality rates from Table II), chi square is found to be 11.04 and P is less than 0.001, indicating the occurrence by chance of such a difference only once in more than a thousand samples of this size.

Effect of vaccination on the incidence of typhus.—The effect of vaccination in reducing the attack rate of typhus was discussed in Gilliam's 1943 report (24). It was not possible to gain further information in 1944 which might have thrown more light on the question. Our data are such that we cannot draw conclusions as to whether multiple doses of vaccine reduce the incidence of typhus or not.

The effect of vaccination during the incubation period of typhus.—There were sixteen patients who first received vaccine 11 days or less before the onset of typhus and one patient who received her first dose 13 days before onset. These patients were considered together in Vaccine Group 4 and have been regarded as vaccinated during the incubation period of typhus. The duration of fever and the clinical severity are shown in Table V, VI, VII, and VIII. It is apparent that the course of illness in the Group 4 cases was somewhat milder and shorter than in the unvaccinated group. One death occurred in Group 4, a 35 year old male patient who was first vaccinated 3 days before onset. The expected number of deaths for a group of seventeen patients with the same sex and age distribution would be 2.6. This difference between expected and observed deaths is not statistically significant.

Duration of the effect of a course of vaccination.—The longest interval observed between the last dose of vaccine and the onset of typhus was 325 days, the next in order being 200, 161, 138, and 117 days. Most of the intervals were less than 100 days. There was no apparent diminution in benefit up to 325 days, but it is not possible to make any statements from these data regarding the maximum duration of the effect of a course of Cox-type vaccine.

DISCUSSION

Before the vaccination program was undertaken, typhus fever among the employees of the Cairo Fever Hospital was characteristically severe. Although few in number, those employees who remained unvaccinated and contracted typhus during the period covered by this study were likewise severely stricken. The contrast between the unvaccinated patients and those who had received two or more doses of Cox vaccine 21 days or more before the onset of illness was very impressive. There were no severely ill patients, i.e., "D", "E", or "F" cases, in Vaccine Group 1 and 2, whereas the evidence indicates that two-thirds of the unvaccinated cases in the same age groups fall into the severe groups, "D", "E", or "F". On the basis of these observations, and insofar as attenuation of clinical course is concerned, it is postulated that "adequate" vaccination against typhus may be defined as two or more doses of

Cox-type vaccine of standard potency[3] administered more than 21 days before the onset of typhus.

There was no sharp division between Group 1 and 2 and Group 3 and 4. One dose of vaccine 21 days before onset seemed to be of value. Indeed, it is possible that one or more doses of vaccine during the incubation period may have a favorable influence on the course of illness. These data cannot be said to establish this point, but the impression is gained that vaccine was of benefit during the incubation period. In this regard Durand (25) makes the following statements about his killed rickettsial vaccine from rodent lungs in reference to epidemic control. "Out of several thousands of doctors and various officials professionally exposed to infection, being vaccinated with three injections of rodent-lung killed classical typhus vaccine only one fatal case occurred while a high mortality rate was encountered among the non-vaccinated personnel. Typhus occurs mostly among the vaccinated when vaccination is done during the incubation period or is over eight months old. The disease, even in the latter two categories is generally mild and of short duration." Further, he states that, using potassium or sodium amylxanthate instead of formol in rodent-lung killed vaccine, "Even a single centimetre seemed to be of value in epidemic control. Typhus fever is nearly always shortened and very mild even when injection has been performed during the last days of incubation."

The figures in the small series in this study would seem to lend support to Durand's conclusions. It is probable that in an epidemic one or two injections of vaccine could be given to a great many of the future victims. The lessening of the severity and the shortening of convalescence would be objectives entirely consistent with the public health aspects of epidemic control. Thus, in view of the data from Vaccine Group 4, we recommend that vaccination be undertaken during an epidemic of typhus and that more information be obtained on the effect of vaccination during the incubation period.

SUMMARY

An account has been given of 61 cases of naturally occurring classical typhus fever in patients who had received, *previous to the onset of illness*, one or more injections of Cox-type vaccine. The course of the disease in the vaccinated has been contrasted with the course in 54 unvaccinated patients.

It is concluded that two or more doses of Cox-type vaccine (1 cc. each) given 3 weeks or more before the onset of typhus reduce the mortality as well as the severity of naturally acquired typhus fever as it occurs under the conditions which prevail during epidemics.

It is regarded as possible that administration of vaccine during the incubation period of typhus will lessen the severity of illness. The authors recommend that vaccination be included in epidemic *control programs*.

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[3] The potency requirements for Cox vaccine are issued to laboratories manufacturing typhus vaccine by the Biologics Control Laboratory of the National Institute of Health, U. S. Public Health Service, Bethesda, Maryland.

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THE INEFFECTIVENESS OF INTENSIVE MAPHARSEN, BISMUTH AND CARBASONE AS CURATIVE DRUGS FOR CHRONIC MALARIA

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Vivax malaria infections acquired by the American serviceman in the South Pacific area are characterized by a tendency to repeated recurrences, and in this respect present a major problem. Some men are now entering their fourth year since becoming infected and are continuing to relapse at almost monthly intervals. Little difficulty has been experienced in interrupting each acute attack, but irrespective of the amount of any drug, or the duration of its administration, it has not been possible to eradicate the infection permanently in those who possess a tendency for repeated relapses. Consequently, in an effort to unearth a curative drug or regime, it seemed wise to explore the effectiveness of some of the arsenical compounds when used in combination with atabrine.

Although it has been well established that neither neoarsphenamine nor mapharsen has much therapeutic effect in *Plasmodium malariae* and *P. falciparum* infections (1, 2, 3) the use of arsenicals has been recommended from time to time for the treatment of *P. vivax* infections, particularly the chronic type (4). However, many of the recommendations were not based on conclusive investigations. Most of the reports have been casual and have emanated from the field, where it has been impossible to differentiate relapses from reinfections and where the therapeutic approach has been to give the arsenical injections at irregular intervals, with an inadequate follow-up.

A search of the literature shows that arsphenamine has been used for many years in the treatment of malarial infections (5, 6). As early as 1910, Werner (7) showed that it had therapeutic value in benign tertian malaria. Since then, this has been corroborated frequently, but it has been stated that relapses following this drug have been common (5). In 1932, Goldman (8), working with mapharsen, not only showed it to be effective in both induced and natural benign tertian malaria, but also reported recurrences following its use to be rare. Very recently, Kay (9), using three doses of mapharsen, to a total of 0.160 grams, in conjunction with atabrine in 67 cases of relapsing tertian malaria, showed that it had no effect on the relapse rate. A similar finding was noted in a review of cases of malaria at this Post where it was found that almost 100 men had received infrequent or interrupted courses of some arsenical. In general the treatments had consisted of from 1 to 5 intravenous injections of neoarsphenamine or mapharsen, administered at various installations, and attended with no apparent beneficial results.

Since this South Pacific vivax malaria is a nonfatal disease, the question may well be asked why use a potentially harmful drug, such as an arsenical, in its treatment. The reasons which led to a trial of intensive mapharsen therapy were:

(1) The failure of any compound thus far used to effect a cure in a large number of men with this type of malaria, i.e., in "chronic relapsers" many of whom will soon be in the fourth year of their infections.

(2) The observation here that all malarial recurrences ceased in a group of eight luetic patients, who were receiving mapharsen therapy (regular 26 weeks course of treatment).

(3) The desire to decide beyond doubt whether mapharsen (as representative of therapeutically effective arsenic compounds) in maximum tolerated dosage would succeed in eliminating the type malaria under consideration and if not, to recommend strongly against its further use.

MATERIALS AND METHODS

The present report includes results obtained in the following treatment programs:

Group I. Mapharsen in combination with atabrine.

Group II. Mapharsen in combination with bismuth.

Group III. Carboarsone and atabrine, and

Group IV. Atabrine alone (control group).

In Group I, the program of mapharsen therapy employed was modelled after the plan suggested by Eagle (10), for the treatment of early syphilis. During an acute relapse of malaria, the arsenical was started simultaneously with atabrine, the

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latter given over a seven day period to a total of 3.2 grams. Mapharsen was given to the patients by intravenous injections 0.06 gram three times a week until each subject had received 20 mgm. per kilogram of body weight. This amount was selected as it represented the smallest quantity of arsenic which could be considered curative in syphilis. The thrice weekly regime was used because, as Eagle found, it provided the least toxic program of intensive arsenic therapy.

The subjects who underwent treatment in this group were carefully selected because many of the men who acquired malaria in the South Pacific are known to be recovering spontaneously. Only those patients were included in this investigation in whom further, regular recurrences (approximately monthly) could be anticipated with a fair degree of certainty (table 1).

Since the use of mapharsen without bismuth is considered to give less satisfactory results in the treatment of syphilis than the two drugs in combination (10), and also since bismuth has some anti-malarial effect, it was decided to try a second and smaller group on this combination, Group II. However, since the majority of subjects in this group were luetics, our program of treatment varied. Five subjects received one weekly injection of 0.2 gram, of bismuth subsalicylate intramuscularly for 12–15 weeks, in addition to the thrice-weekly injections of mapharsen as in Group I. Four others received regular anti-luetic therapy. Seven of the men in this group received a minimum of 30 mgm. of mapharsen per kilogram of body weight (table 2).

In Group III, 48 patients received carbarsone orally. The basis for its trial was that organic pentavalent arsenicals are more active against protozoan forms than are the organic trivalent arsenic compounds such as mapharsen (11). According to Bispham, acetarsone, a related compound has been tried, and, although it, too, controls the acute P. vivax infection, it does not prevent relapse. Acetarsone, however, has only one-eighth the therapeutic index of carbarsone, so it was felt that the latter deserved a trial. Although our carbarsone treatment schedule could not be considered intensive, it did afford the opportunity to use an oral drug containing penavalent arsenic.

To 10 men in this group, only carbarsone, 0.5 gram, twice daily, was administered for 7 days. In the remaining 40 cases, carbarsone, in the same dosage as above, was given concurrently with atabrine, 0.2 gram, twice daily for seven days (total dose 2.8 grams). Subjects in this group were not selected as in Groups I and II but represented 48 successive cases admitted to our wards with acute episodes of malaria. This was similar to the selection of cases in the control Group (IV) treated with atabrine (0.2 gram tid for 1 day and bid for 6 days to a total dose of 3.0 grams).

Accepted procedures were followed in an effort to detect early evidence of toxic reactions during the period of actual treatment with mapharsen. In Groups I and II during therapy and for a period of 3 months following completion of the therapy, each subject was followed by means of a weekly blood smear for malaria parasites and a urine examination to determine whether he was treating himself with quinine or atabrine. Furthermore, if at any time any of these men complained of feeling sick, additional blood smears were examined.

In the Groups III and IV following the completion of therapy blood smears of all subjects were examined twice a week for a period of three months.

TOXIC REACTIONS

The men in Groups I and II suffered no serious toxic reactions to the arsenic administered. One patient developed an acute psychosis, which, in part, might have been the result of mapharsen, although there were several other factors concerned. He made an uneventful, spontaneous recovery. Gastric distress was encountered ten times, some aching (often of the head) with and without fever was observed six times, diarrhea was complained of five times and thrombosis or local tissue reaction was seen in three of the patients. Two subjects showed a transient elevation of blood bilirubin to 2.6 and 2.8 mgm. per cent respectively.

In the carbarsone group, no toxic effects were noted.

RESULTS

There were 19 patients in Group I who received mapharsen and atabrine, four were dropped as shown in table 3. Of the 15 who completed treatment, none exhibited clinical or parasitic relapses while under treatment and all were improved subjectively. Within one month after the cessation of treatment seven of the 15 had both parasitic and clinical relapses and by the end of the three months observation period only three had remained parasite or clinically free of malaria (table 1).

TABLE 1

Effect of mapharsen and atabrine on chronic Vivax malaria

Group I. Mapharsen, 0.06 gram 3 times a week. Total of 20 mgm. per kilo of body weight

SUBJECT	NUMBER OF RELAPSES	LAST 3 ATTACKS OF MALARIA BEFORE ONSET OF THERAPY	MAPHARSEN					RELAPSE DURING 3 MONTHS FOLLOW-UP	
			Started Date	Completed Date	Total number of doses	Total amount	During therapy	Parasite date	Clinical date
						gms			
E. G. B.	20	Oct. Dec. Jan.	1/29	3/19	21	1.08	0	4/8	4/8
C. E. G.	12	Oct. Nov. Jan.	1/12	2/28	21	1.20	0	0	0
J. M. G.	15	Oct. Nov. Jan.	1/17	3/14	23	1.36	0	3/28	3/31
J. F. G.	20	Sept. Nov. Jan.	1/12	4/11	29	1.54	0	4/18	4/23
D. W. L.	30	Nov. Dec. Jan.	1/29	3/19	22	1.24	0	5/23	6/12
K. R. M.	9	Sept. Parasitemia through Dec. and Jan.	1/5	3/7	25	1.33	0	0	0
J. J. N.	15	Oct. Dec. Jan.	1/22	3/23	27	1.56	0	4/4	4/6
R. C. N.	15	Sept. Nov. Jan.	1/15	3/2	21	1.14	0	4/11	4/11
A. D. O.*	10	Sept. Nov. Jan.	1/6	3/16	29	1.68	0	0	0
J. N. P.	10	Sept. Nov. Jan.	1/10	2/28	22	1.23	0	3/14	3/19
W. L. Q.	20	Dec. Jan. Jan.	1/29	3/23	23	1.28	0	4/18	4/20
F. H. R.	20	Sept. Dec. Jan.	1/22	3/30	26	1.50	0	6/13	6/13
I. L. S.	10	Oct. Dec. Jan.	1/17	3/21	26	1.47	0	6/13	0
G. A. S.	4	Oct. Nov. Jan.	1/10	3/12	28	1.46	0	4/23	4/23
A. G.	15	Nov. Jan. Mar.	3/2	5/4	23	1.35	0	5/16	5/20

* Had parasite and clinical relapse 8/8.

TABLE 2

Effect of mapharsen and bismuth on chronic Vivax malaria

SUBJECT	NUMBER OF RELAPSES	MAPHARSEN		TOTAL GMS. MAPHARSEN	TOTAL GMS. BISMUTH	DURING THERAPY	RELAPSE DURING 3 MONTHS FOLLOW-UP		COMMENT
		Started Date	Completed Date				Parasite date	Clinical date	
1. E. W. B.	8	2/ 2/45	4/ 4/45	1.5	2.0	0	4/25/45	6/17/45	Non-luetic
2. G. H. D.	12	1/31/45	4/13/45	1.8	2.4	0	0	0	Non-luetic
3. P. A. C.	5	5/20/44	8/30/44	1.95	3.2	0	0	0	
4. R. K.	11	1/22/45	4/13/45	1.90	3.6	0	7/ 5/45	7/ 6/45	
5. J. W. M.	10	3/ 7/45	6/ 1/45	2.1	3.0	0	7/11/45	7/12/45	
6. A. W. K.	17	10/23/44	5/ 2/45	2.4	3.2	3/14/45	5/19/45	5/19/45	At time of 1st relapse, had received mapharsen 1.2 gm., bismuth 1.0 gm.
7. J. D.	24	7/15/44	2/28/45	2.4	4.4	0	0	0	
8. T. D. B.	22	9/11/44	3/ 9/45	2.4	3.2	0	0	0	
9. W. B.	21	2/27/44	3/28/45	1.8	3.6	2/ 9/45	0	0	At time of relapse, had received mapharsen 1.8 gm., bismuth 3.6 gm.

1–5 Received Mapharsen 0.06 gm. 3 times a week and Bismuth 1.2 gm. I.M. 1 time a week for 8–12 weeks.
6–8 Received regular "26 weeks course" of anti-luetic therapy.
9 Received 12 months course of anti-luetic therapy.
Note: All luetic patients (3–9) at completion of above treatment showed reversal of blood Kahn.

Similar results were observed in the nine patients in Group II who received mapharsen and bismuth (table 2), although two men relapsed while under treatment. It is worthy of note, however, that both of these relapses occurred during a course of bismuth, one had received a total of 1.2 grams, of mapharsen, but none for six weeks prior to the attack, while the other had received 1.8 grams, of mapharsen, but none within two weeks of the attack. Three other subjects relapsed within three months of completion of treatment. In all, only four patients remained free of a clinical or parasitic relapse (table 2).

Carbonsone given without atabrine was found to terminate the attack in five of ten men in Group III by the end of 24 hours, in two by the end of 48 hours, in one by 72 and in two by the end of 96 hours. Parasites had disappeared from the peripheral blood during treatment in all but one of these patients. In four cases, however, parasitemia recurred within ten days. In the three month period all ten cases had parasitic relapses but only three experienced clinical attacks. In the group of 38 who received atabrine in conjunction with carbarsone, at the end of the three month period, there were 24 parasitic relapses (63%) and 14 clinical relapses (37%). In comparison, the control group of 94 patients treated with atabrine alone had 66% parasite and 47% clinical relapses (table 3).

TABLE 3

Relative effectiveness of mapharsen, bismuth, carbarsone and atabrine

	Total number treated	Relapsed within 1 month		Relapsed within 1–2 months		Relapsed within 2–3 months		Total relapses within 3 months		Treatment stopped prior to completion of course
		Parasite	Clinical	Parasite	Clinical	Parasite	Clinical	Parasite	Clinical	
Group I. Mapharsen and Atabrine	19	7 47%	7 47%	2 13%	2 13%	3 20%	2 13%	12 80%	11 73%	1 diarrhea 4–1 psychosis 2 uncooperative
Group II. Mapharsen and Bismuth	12	4 44%	3 33%	0	0	1 11%	2 22%	5 55%	5 55%	1 g i distress 3–1 uncooperative 1 transferred
Group III-A. Carbarsone alone	10	8 80%	3 30%	0	0	2 20%	0	10 100%	3 30%	
Group III-B. Carbarsone and Atabrine	38	5 13%	4 11%	8 21%	6 16%	11 29%	4 11%	24 63%	14 37%	
Group III. Carbarsone (total)	48	13 27%	7 15%	8 17%	6 13%	13 27%	4 8%	34 70%	17 35%	
Group IV. Atabrine	94	10 11%	3 3%	40 43%	25 27%	12 13%	16 17%	62 66%	44 47%	

CONCLUSIONS

1. Arsenic therapy administered intensively by intravenous injections with or without bismuth or atabrine has no influence on the relapse rate of South West Pacific vivax malaria.

2. Although carbarsone exerts a therapeutic effect on the acute attack of vivax malaria, it is definitely inferior to atabrine.

3. In view of the above findings, arsenical preparations such as mapharsen and carbarsone are not indicated in the treatment of acute or chronic malaria.

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PIGMENTATION OF THE PALATE AND SUBUNGUAL TISSUES ASSOCIATED WITH SUPPRESSIVE QUINACRINE HYDROCHLORIDE THERAPY

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A slate grey pigmentation of the hard palate and the subungual areas of the fingers and toes has been observed among the troops serving in the South West Pacific Area. The purpose of this investigation is to determine the prevalence and etiology of this condition.

In the hard palate, the pigment deposits vary in intensity and color from slate-grey to blue-black, first appear near the midline, and extend laterally to the gingival margins. There is usually a well-defined border. Several patients who had partial upper dentures had pigment deposited only in those areas untouched by the prosthesis. In the subungual areas, the early lesion is a slate grey patch or transverse line near the center of the nailbed. The pigmentation spreads and intensity increases but does not involve the distal quarter of the nailbed. In the most advanced cases almost the entire subungual tissue may be involved and there is direct extension into the adjacent skin along the lateral borders of the nails. The nails are unaffected. There are no associated symptoms and the average soldier is not aware of the condition until it is called to his attention.

Extensive reviews of the literature on pigmentation, such as that of Jeghers (1), fail to mention this syndrome. Subungual pigmentation occurring in two per cent of a Marine unit serving in the South Pacific is reported by Barr (2). Pigmentation of the hard palate is not mentioned. He attributes the condition to continuous suppressive quinacrine (atabrine) therapy on the basis of his observation that the pigmentation diminishes when administration of the drug is discontinued for one month.

RESULTS

Five hundred men selected at random among medical and surgical patients in a general hospital in the South West Pacific were examined by two observers. Length of time in the tropics, duration of suppressive quinacrine therapy,[3] occurrence of slate pigmentation of the palate and subungual tissues and history of attacks of malaria were recorded. Pigmentation of some degree occurred in 156 or 31.2 per cent.

The incidence of slate pigmentation in patients who had been on suppressive quinacrine therapy and in the tropics for from 1 to 29 months is shown in table 1. Frequency is calculated for each 6 month period. In no instance was pigmentation observed in patients who had received suppressive quinacrine therapy for less than 7 months. Thereafter the incidence increased to a level of 63.8 per cent for the 19 through 24 month period and 63.6 per cent for the 25 through 29 month period.

Since no patient who had been on suppressive quinacrine therapy less than 7 months showed any slate pigmentation, it was of interest to find how long the 146 patients in this group had been in the tropics. Seventy had resided in the tropics more than 6 months but had been on islands where there is no malaria and suppressive quinacrine is not taken. This group of 70 may be contrasted with the group of 354 patients who also had been in the tropics for more than 6 months but who had taken quinacrine for from 7 to 29 months. In the latter group 156 (44.0 per cent) showed slate pigmentation of some degree. If tropical residence were a causative factor, some of the first group should have exhibited pigmentation. The absence of any involvement in this group of 70 on the basis of chance alone is calculated to occur considerably less often than once in a million times.

Three patients with marked pigmentation were observed for a period of 6 weeks during which no quinacrine was given. In no instance was reduction in its intensity or extent observed. There was found to be no correlation between a history of malaria and the incidence of pigmentation.

The red blood count, white blood count and hemoglobin of these patients were not abnormal

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[3] The majority received 0.1 gram of quinacrine hydrochloride daily but in some units the dose was increased to 0.5 gram twice weekly for a period of several months.

except in those whose presenting disease, for which they were hospitalized, caused abnormalities. The urine of three patients with marked pigmentation was examined for porphyrin by ultraviolet light. None was present in 24-hour specimens. The urine of 30 others was examined for hemosiderin with negative results (3).

PATHOLOGY

The nature and distribution of the pigment was studied by biopsy. Sections were obtained from the mucous membrane of the hard palate in two patients and from the skin on the lateral aspect of the toe, adjacent to the nail, in another. In both locations, there is extensive deposition of a pigment which stains yellow-brown with hematoxylin-eosin. This pigment is most abundant immediately beneath the epidermis but is also scattered through the deeper layers of the derma and lamina propria. It is found in the mononuclear phagocytic cells and extracellularly. None could be demonstrated in the epithelium.

TABLE 1

Relation between suppressive quinacrine therapy and occurrence of pigmentation

	MONTHS ON SUPPRESSIVE QUINACRINE				
	0–6	7–12	13–18	19–24	25–29
Number in group	146	161	124	47	22
Number showing pigmentation	0	44	68	30	14
Per cent showing pigmentation	0	27.3	54.8	63.8	63.6

In an effort to determine the chemical nature of the pigment, other stains were employed. With Gomori's modification of Turnbull's blue method for hemosiderin (4), the characteristic blue reaction is observed. Mallory's hematoxylin stain for iron and copper, on tissues fixed in formalin, stains the pigment a deep brown. These reactions suggest that the pigment contains iron in an accessible form and is hemosiderin.

Sections from the skin adjacent to the toe nail also reveal branching, thin, somewhat granular, delicate filaments in the derma and epidermis, with some extension beyond the margins of the tissue, and yeast-like forms singly and in clumps. Fungi are not demonstrated in sections from the palate. They are believed to be contaminants but are mentioned because further studies may show that they are of importance in localization of the pigmentation.

DISCUSSION

The prevalence of palatal and subungual pigmentation among troops who have served more than six months in the South West Pacific Area is demonstrated in this survey. Previous to Barr's report, this condition had not been described among persons living in the tropics. Our analysis does not suggest that tropical service alone is a contributing factor. There is positive correlation with quinacrine consumption.

Assuming that the pigment is hemosiderin, neither its derivation nor the factors which determine the sites of deposition are known. There is no evidence of previous hemorrhage or excessive blood destruction. Recent studies on the toxic effects of excessive doses of quinacrine in experimental animals may be significant. Wright and Lillie (5) report hemosiderosis of the spleen and lymph nodes in association with more extensive deposition of non-ferrous pigment in the intestinal mucosa, lymph nodes, spleen, liver, renal glomeruli and medullary tubules, an interstitial and exudative monocytic pneumonia, a focal myocarditis and myositis, and often portal thrombi and hepatic infarcts. Martin, Cominole and Clark (6) also demonstrated extensive pathological changes after administration of excessively large doses but when smaller doses were given over a longer interval, the only finding was "hemosiderin-like" pigment in the liver and spleen. The detailed studies of Siegel and Mushett (7) cannot be summarized adequately here. They conclude that the pathologic alterations definitely attributed to the toxic doses of quinacrine are large necrotic foci in the right side of the liver, necrosis and fibrosis of the myocardium and voluntary muscle and changes in the cortex of the adrenal gland, the reticuloendothelial system and the kidney. Some of the animals which survived a single large dose for several weeks had macrophages in the liver, spleen and lymph nodes which contained iron-staining pigment but as this was found also in some of the control animals, it was considered of questionable significance.

CONCLUSIONS

1. Deposition of a slate grey pigment in the hard palate and subungual tissues of troops in the South West Pacific Area is described.

2. Pigmentation is not observed among troops who have been on suppressive quinacrine therapy for less than 7 months but occurs with increasing frequency among those who have taken quinacrine over a longer interval.

3. Tropical service alone is not a significant factor in the etiology of this condition.

4. Histological studies show that the pigment contains iron in an accessible form and may be hemosiderin.

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COMPLEMENT FIXATION IN RELAPSING PLASMODIUM VIVAX MALARIA[1]

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An unusual opportunity for study of complement fixation in relapsing *Plasmodium vivax* malaria, acquired in Pacific war areas, was afforded us at Kennedy General Hospital. We were able to follow the complement fixation reactions for malaria over a period of 6 months in a group of such patients, and to correlate the findings with coincident blood films and with clinical activity.

These studies, which are described below, show that in relapsing *vivax* malaria:

Complement fixation is a more sensitive method of detecting persistent infection than is the examination of a coincident thick blood film.

Complement fixation is an expression of infection, and is directly related to existent or recent parasitemia, which may, or may not, have been of sufficient level to produce a clinical attack.

A consistently positive complement fixation for malaria continuing for more than 2 months is highly indicative of eventual relapse. The over-all incidence of positive complement fixations increases with relapse and continues to increase after termination of the attack.

A series of negative complement fixation reactions accompanied by negative blood films suggest the possibility of clinical cure, provided such observations are obtained over a sufficiently long time period. This period has not been determined, but it is more than six months.

MATERIALS AND METHODS

Patients

The data collected include observations on 394 patients divided into 2 groups which are designated Series A and Series B.

Series A included 133 patients who were studied during the 6 months' period ending 31 December 1943. In this group, 287 coincident complement fixation tests and blood film examinations were performed. Forty-five patients were tested only once; 34, two times; 22, three times; 17, four times; 14, five times; while 6 successive samples of blood were obtained from 1 patient.

Series B included 261 patients on whom 6,220 complement fixation tests were performed. Of these patients, 199 were followed for the entire 6 months' period, while 62 patients were studied for periods ranging from a few weeks to 100 days.

All of the patients in both series were ambulatory except for brief periods of malaria illness.

All were free of syphilis, a most important consideration since we have found that certain high-titered syphilitic sera may give nonspecific reactions with malaria antigen, just as sera from malarious patients may react with the Wassermann or Kahn antigens.[2]

Collection of sera

Blood was drawn for serological tests from patients in Series A at intervals of 2 weeks; from patients in Series B at intervals of 3 or 5 days. The blood samples were stored in the refrigerator overnight and the sera removed the following morning. No preservative was added to the sera, and all were tested within one week after collection.

Blood films

Thick blood films were made routinely at the time of drawing blood for all serological tests. Daily thin blood films were also made on patients in Series B for 15 days following the onset of attack and institution of treatment. Giemsa's stain was used, and enumeration of parasites was made when-

[1] The studies on which this paper is based were supported by a grant from the Tennessee Valley Authority through the Division of Preventive Medicine, University of Tennessee College of Medicine.

[2] It has been stated elsewhere (Dulaney, Stratman-Thomas and Warr 1942) that false positive complement fixation using malaria antigen is rarely found with serum from known syphilitics with positive syphilis complement fixation. This statement was based on water bath incubation and is still believed to be true when this technique is employed. When ice box fixation is used, nonspecific positives are obtained with malaria antigen and syphilitic sera. This problem is now under study.

ever the density was sufficient. Blood films from Series A were examined in the Preventive Medicine laboratory, University of Tennessee; those from Series B in the Kennedy General Hospital laboratory.

Criteria of attack

The criteria of an attack of malaria were a body temperature of 100°F., or over, accompanied by demonstrable parasitemia. Sporadic parasitemia during the interattack periods constituted a parasitic but not a clinical relapse.

Antigens

A single lot of dried *P. knowlesi* parasites, a pool from 3 monkeys, was used for preparation of antigens used in Series A. Another pool of dried parasites from 6 monkeys was used for all tests in Series B. The method of harvesting and treatment of parasites has been described (Dulaney, Stratman-Thomas, and Warr, 1942).

Saline extracts of the dried parasites were employed for all tests in Series A and for approximately one-fourth of the tests in Series B. A phosphate buffer extract (Dulaney and Morrison, 1944) was used for the remaining tests after 300 comparative tests had proved its potency.

Antigens were frequently checked by titration with sera of known antibody content. Both saline and phosphate buffer extracts were stored in the freezing unit of the refrigerator and a sufficient quantity of antigen for 1 to 2 days' tests was thawed, and centrifuged to clear of precipitated pigment immediately before use.

Complement fixation procedure

The routine complement fixation procedure consisted of a two-tube qualitative test. Titrations for antibody titer were carried out on 235 serum samples of Series A. Certain patients of Series B were selected and their sera titrated at intervals. In many instances there was not sufficient serum for titration and the method of collection and initial handling of sera made storage impractical. Anticomplementary sera were titrated whenever possible.

The modified micro-Kolmer technique (Dulaney and Morrison, 1944) was used for all tests in Series A and for approximately one-third of the tests in Series B.

The procedure recommended by Rein, Bukantz and Kent of the Army Medical School for use at Kennedy General Hospital was employed for testing the remaining sera, after 500 comparative tests had been made. This procedure included titration of complement and hemolysin according to Maltner, Maltner and Wadsworth (1939); namely, the determination of the 50 per cent hemolytic dose of complement by a colorimetric method and use of the optimal amboceptor unit. The test proper consisted of combining 0.1 cc. of serum, 0.2 cc. of antigen, 0.1 cc. of physiological saline solution, and 0.2 cc. of complement containing four 50 per cent units. Antigen controls containing 2 doses of antigen and complement deterioration controls were included. After overnight incubation in the refrigerator, 0.4 cc. of sensitized sheep red cells were added and readings made after a second incubation period of 30 minutes at 37°C.

SEROLOGICAL PATTERNS

Patients with relapsing *vivax* malaria varied greatly in their production of complement fixing antibodies. The findings were similar to those described by Coggeshall and Eaton (1938) for monkeys with chronic malaria.

The 199 patients observed for the entire 6 months' period were classified as to serological pattern in the following manner:

I. Positive pattern

This group of 89 patients gave continued positive reactions with not more than 3 scattered negative reactions throughout the period of observation. The maintenance of this high state of antibody production was associated in most instances with frequent or infrequent positive blood films or with attack. A few patients continued to give 4+ reactions with no demonstrable parasitemia or attack. It is our opinion that such persistence of a positive serological state is due to a constant state of infection which may or may not have been demonstrable by blood film but which continued to afford stimulation for antibody production. This opinion is substantiated by the high incidence of relapses in this group.

II. Negative pattern

Thirty-eight patients gave continued negative reactions with not more than 3 scattered positives throughout the 6 months' period of observation. These patients formed 3 subgroups; namely, (a) those who remained free of attack, who gave consistently negative serological reactions along with

negative blood films; (b) patients who showed very frequent parasitemia in addition to attacks; and (c) those individuals who experienced 0 to 2 attacks with only occasional parasitemia. These subgroups represent, in order given, individuals who are probably approaching clinical cure, individuals unable to form antibodies, and individuals producing antibodies in concentrations too low to be detected.

III. Changing pattern

The remaining 72 patients exhibited changing serological patterns as follows:

(*a*) *Negative to positive.* Eleven patients showed a series of negative reactions followed by a series of positive reactions. The change in serological pattern was associated with a positive blood film or attack.

(*b*) *Positive to negative to positive.* Eight patients gave a series of positive reactions associated with positive blood film or attack, which was followed by a series of negative reactions upon treatment. In turn, these negative reactions were followed by a series of positive reactions which were associated with positive blood film or attack.

(c) *Positive to negative.* In 17 instances a series of positive reactions associated with positive blood film or attack was followed by a series of negative tests. This change occurred spontaneously in some cases; in others it was correlated with treatment.

(*d*) *Negative to positive to negative.* In 14 patients a series of positive reactions, associated with sporadic parasitemia or attack, followed a series of negative reactions. Following treatment the positive pattern gave way to a series of negative reactions.

(*e*) *Indeterminate.* We did not classify 22 patients because of the frequent fluctuations in the serological patterns exhibited.

RESULTS

In previous studies (Dulaney, Stratman-Thomas, and Warr, 1942) the principal interest was the determination of the diagnostic possibilities of the complement fixation test for naturally acquired malaria. It was found that 81.6 per cent of 125 patients with demonstrable parasites gave positive reactions with *P. knowlesi* antigen.[3] These individuals constituted part of a group of clinic and hospital patients presenting symptoms which warranted examination of blood films for malaria parasites and in this sense they were unselected.

In the present study we are dealing with a selected group of patients in that all have more or less well-documented histories of repeated attacks of *P. vivax* infection. All had received suppressive atabrine treatment. We are therefore interested chiefly in the ability of the complement fixation test to detect persistent subclinical infection, to offer some reliable information for future management of these individuals. Comparison of complement fixation reactions and coincident blood film examinations indicates that the serological test is the more sensitive procedure in detecting such infections.

There were 287 coincident complement fixation and blood film examinations on the 133 patients in Series A and 6,220 such concurrent tests on the 261 patients in Series B. Table I shows the distribution of these tests for the entire group of 394 patients.

TABLE I

Distribution of 6,507 coincident complement fixation tests and blood film examinations from 394 patients with P. vivax infections

BLOOD FILM	COMPLEMENT FIXATION		
	Positive	Negative	Total
Positive	577 (9%)	277 (4%)	854 (13%)
Negative	3,430 (53%)	2,223 (34%)	5,653 (87%)
Total	4,007 (62%)	2,500 (38%)	6,507 (100%)

χ^2—15.45. P—<0.0001.

As shown in Table I, 4,007 of the 6,507 complement fixation tests were positive in contrast to 854 of the 6,507 blood films. The over-all sensitivity of the complement fixation reaction was therefore 62 per cent when the corresponding sensitivity of the blood film examination was 13 per cent.

When the two tests are compared directly, it is again evident that the diagnostic sensitivity of the complement fixation test in relapsing malaria is greater than that of the routine thick blood film: 4,007 (62 per cent) positive complement fixations were accompanied by 577 (9 per cent) positive blood films. There were 6.9 times as many coincident positive complement fixation reactions as

[3] Only one examination per patient.

positive blood films. There was agreement in the results of 2,800 (43 per cent) complement fixation and coincident blood film examinations. The 3,707 discrepant results included 3,430 (92 per cent) positive complement fixation reactions in contrast to 277 (7.5 per cent) positive blood films.

Complement fixation is an expression of infection and is directly related to existent or recent parasitemia.

Data regarding this question were obtained entirely from the 199 patients of Series B who were followed for the full 6 months' period (Table II).

TABLE II

Showing correlation between clinical illness, positive blood films, and complement fixation reactions in 199 patients observed for six months

Number of attacks	Number of patients	Blood film		Complement fixation		Cf/bf ratio
		Total positive	Positive per patient	Total positive	Positive per patient	
0	46 (23%)	122	2.65	419	9.11	3.43
1	80 (40%)	284	3.55	1,470	18.38	5.18
2	46 (23%)	199	4.33	994	21.61	4.99
3	24 (12%)	126	5.25	609	25.38	4.83
4 and over	3 (2%)	22	7.33	42	14.00	1.91
Total	199 (100%)	753	3.78	3,534	17.76	4.69

Those 199 patients were grouped as to number of attacks experienced during the 6 months' observation period. Forty-six patients had no attack; 80 had 1 attack; 46 suffered 2 attacks; while 24 individuals experienced 3 periods of clinical activity. Four attacks were recorded for each of 2 individuals and 5 attacks for 1 patient. The average number of positive tests per patient is recorded and the complement fixation-blood film ratio determined.

The close correlation between attack, positive blood film, and positive complement fixation is apparent. The incidence of positive blood films rises steadily with the attack rate. The rate for the group experiencing 3 attacks is twice that for the group showing no attack. The rate for the one individual experiencing 5 attacks is almost 3 times that of the first group. Parenthetically it may be pointed out that the actual number of blood films recorded appears small because (1) the routine observations made at 3- or 5-day intervals sometimes failed to coincide with the attack day, and (2) institution of treatment on the day of attack often changed a positive blood film to a negative within 24 hours.

The incidence of positive complement fixation tests likewise rose with the attack rate except in the patients having four or more attacks. Here the average number of positive complement fixation tests declined while the coincident blood films increased. Even though these groups are small, it is believed that this finding is significant in that it indicates either the circulating antibodies are removed or the antibody formation inhibited in these patients so frequently experiencing parasitemia and relapse. As would be expected the complement fixation-blood film ratio is lowest in these groups, and next lowest in the group showing no attack, which included patients of negative pattern.

The close relationship between complement fixation, attack, and parasitemia invites the question as to the possible usefulness of the complement fixation test in predicting relapses. Can complement fixation serve as a basis for segregation of individuals with a high probability of relapse from those with slight probability of relapse? We believe a tentative division to be possible if the prediction is based on a series of reactions rather than on isolated tests. The data in Table III indicate that positive reactions continuing for more than 2 months are highly prognostic of eventual relapse.

Observations were again restricted to the 199 patients tested at intervals of 3 or 5 days for the entire six months' period. These patients were grouped as to serological pattern. It was found that 89 patients fell into Group I, the positive pattern group; 38 into Group II, the negative pattern group; and 72 into Group III, made up of individuals with changing serological pattern. These groups are not large, but it is believed that the long term studies afford highly significant results.

Obviously the most informative data are to be derived from Groups I and II which constitute 127 (63.8 per cent) of the 199 patients. Eleven (12 per cent) of patients in Group I did not relapse during the observation period even though they maintained a consistently positive serological pattern,

while 78 (88 per cent) of this group relapsed from 1 to 4 times. The ratio of relapse to nonrelapse is 7:1.

Group II, made up of 38 individuals who maintained negative serological patterns (not more than 3 scattered positives) throughout the period of observation, included 22 patients (58 per cent) who did not relapse, and 16 (42 per cent) who relapsed 1 to 5 times (9 had 1 relapse, 5 had 2, 1 had 3 and 1 had 5). The ratio of relapse to nonrelapse is 0.72:1.

Under the usual conditions of infection the complement fixation reaction for malaria becomes negative within 1 to 2 months after attack and institution of treatment, and remains negative unless the patient relapses, when it again becomes positive.

TABLE III

Relation between serological pattern and relapse in 199 patients observed for six months

GROUP	NO RELAPSE	1 OR MORE RELAPSES	TOTAL PATIENTS
1	11 (12%)	78 (88%)	89 (44.7%)
2	22 (58%)	16 (42%)	38 (19.1%)
3	15 (21%)	57 (79%)	72 (36.2%)
All groups.....	48 (24%)	151 (76%)	199 (100%)

χ^2—30.67. P—<0 000001.

If such a patient relapses, when all possibility of reinfection is ruled out, it is apparent that he has remained infected even though the parasites have been too few to be detected in blood films or to provide a stimulus for antibody formation. Patients who maintain a positive complement fixation reaction for periods of 2 months or longer after attack and treatment may be accepted, in most instances, to harbor substantial numbers of parasites, presumably in the reticulo-endothelial system. These parasites not only provide the stimulus for continued antibody formation but may increase to levels sufficient to initiate a clinical attack. It is interesting that only 3 (27 per cent) of the 11 patients of Group I who did not relapse gave persistently negative blood films while sporadic parasitemia was demonstrated for 8 (73 per cent). It is our belief that these 8 patients are likely to relapse.

The 22 patients of Group II who did not relapse during the 6 months' period included 16 (73 per cent) who gave consistently negative blood films and 4 who had only 1 positive blood film recorded.

The average interval between attacks experienced by the patients was approximately 2 months. Thus, patients who continue to give positive complement fixation reactions for longer than 2 months after attack and treatment will probably relapse.

Group III included 72 (36.2 per cent) of the 199 patients. Seventy-nine per cent (57) of these individuals relapsed at some time during the 6 months' period. All gave some positive complement fixation reactions. In 21 patients there were records of consistently positive complement fixation reactions, in 12 patients consistently negative reaction, for a period of 2 months before relapse.

The relation of continued positive complement fixation to relapse is further demonstrated in figure 1, which depicts the over-all trend of positive complement fixation and blood films for the 167 individuals experiencing 274[4] attacks during the period of study. The observations were extended whenever possible to include periods of 25 days before and after attacks. The records for each 3 days are grouped together and the percentage of positive complement fixation reactions determined. It is evident that the over-all incidence of positive complement fixation for relapsing patients remains high before and after the attack day even though individual records give beautiful demonstrations of negative reactions prior to relapse followed by a series of positive reactions, a situation described by Coggeshall and Eaton (1938) for chronically infected monkeys. There is a steady rise in the over-all incidence of complement fixation beginning approximately 2 weeks before the day of attack. The attack day is followed by an increased incidence of positive reactions. Seventy-four per cent of positive complement fixation reactions are recorded on day of attack, but the incidence rises to 80 per cent within 3 days after the attack, and this level is maintained for some time.

The incidence of positive blood films also increases before attack but drops precipitously after institution of treatment. Three days after the attack day the ratio of positive complement fixation to positive blood film is 16:1. Six days after the attack day this ratio is 80:1. The positive complement fixation may continue indefinitely,

[4] Figures from the entire group of Series B.

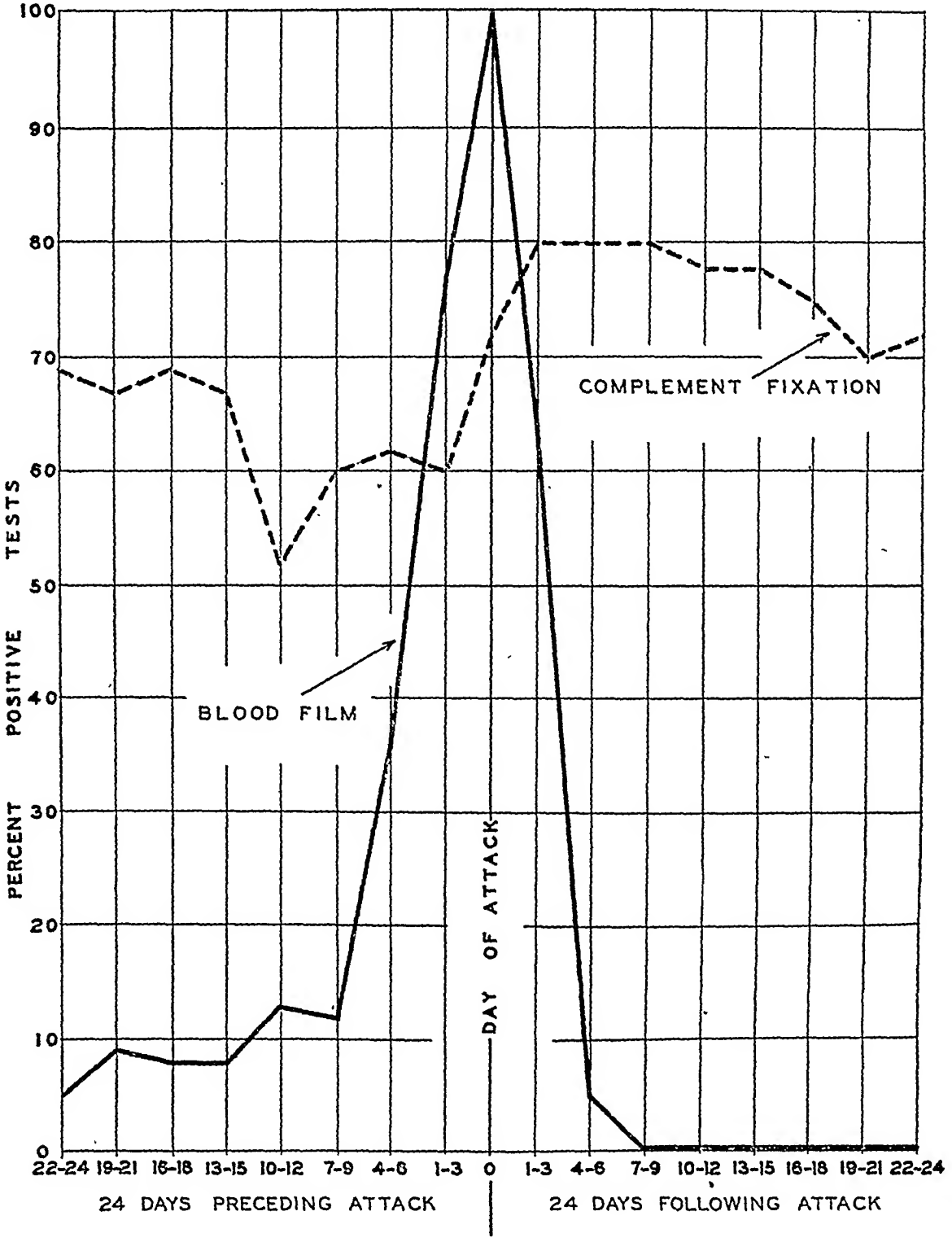

FIG. 1. THE TREND OF POSITIVE COMPLEMENT FIXATION TESTS AND POSITIVE BLOOD FILMS, FOR 167 INDIVIDUALS EXPERIENCING 274 ATTACKS, IN RELATION TO THE DAY OF ATTACK

steadily decline, or decline and rise again with subsequent parasitic episodes. The great variation in individual patterns is expressive of differences in the antibody response to antigen or to relative quantities of antigen and antibody present in the blood stream.

Another interesting question concerns complement fixation and clinical cure. Can clinical cure be predicted on serological grounds? We believe that continued negative complement fixations accompanied by negative blood films offers good prognosis of clinical cure providing these observations are extended over a sufficiently long time period. That time period has not yet been determined, but it is evidently more than 6 months since 2 of the negative pattern patients relapsed at the very end of the study period. (These patients had not given consistently negative blood films.) Thirteen individuals (6.5 per cent of the group of 199) admitted to the study on the basis of attacks occurring late in 1943, went through the entire 6 months' period with no subsequent clinical activity, no positive blood film, no positive complement fixation reactions. The prognosis for their continued freedom from *vivax* disease would appear to be good. On the other hand we have records of individuals exhibiting frequent relapse and even more frequent parasitemia who gave consistently negative serological reactions. Five such patients may be mentioned. These had a total of 12 attacks, 62 positive blood films, and 172 negative complement fixation reactions. These patients have a poor prognosis and their negative serological state would seem to be due to lack of formation of antibodies at detecable levels, since their parasite counts were never high. The 62 positive blood films represent 7.3 per cent of the total number of positive blood films recorded.

It would appear that the formation of antibodies, demonstrable by the complement fixation test, is beneficial in the sense that the best prognosis is found in the patient who develops a high antibody titer, who is able to cope with infrequent or even frequent parasitemias of low levels with little clinical activity, and who eventually becomes serologically negative and remains so.

DISCUSSION

Individuals returning from war areas where malaria is endemic present a challenge to the diagnostic laboratory. Many of these individuals have experienced multiple attacks of malaria, and they have received suppressive drugs as well as prompt treatment upon attack. In this group are individuals who are able to cope with mild, infrequent parasitemias without clinical manifestations. The antigenic stimulus is at the same time sufficient to maintain a consistently high state of antibody formation. In others the frequent parasitemias and relapses either inhibit antibody formation, remove antibodies from the circulating blood, or reduce them to a very low level.

Complement fixation can be used to advantage in examination of these individuals since it will detect continued infection in a high percentage of cases, give tangible information as to expected relapse, and possibly offer prognosis of clinical cure.

The complement fixation test for malaria has undoubtedly suffered from early overenthusiasm regarding its practical possibilities. Too much has been expected of its sensitivity as a routine laboratory procedure. Objections to its specificity have been made because of the nonspecific reactions with syphilitic sera. As a matter of fact, the sensitivity of the complement fixation test for malaria compares very favorably with the over-all sensitivity of the serological tests for syphilis. Its specificity is undoubtedly decreased by the cross reactions with high-titered syphilitic sera but to no greater extent than is the specificity of the Wassermann reactions in malarious individuals.

The accepted method of laboratory diagnosis of malaria is by blood film examination. Success of this method depends upon (1) the time of collecting blood samples, (2) the technique of preparation and staining of blood films, and (3) the ability of the examiner. Repeated careful examination of blood films is often necessary to establish a diagnosis of malaria. A satisfactory examination of a negative blood film requires at least 10 minutes' study. Parasites are difficult to demonstrate in blood films after institution of treatment: it is often impossible to demonstrate them within 24 to 48 hours after administration of anti-malarial drugs.

The success of a complement fixation test likewise depends upon certain conditions such as (1) time of collection of blood samples, (2) technique, and (3) antigen of proved specificity. The *P. knowlesi* antigen is of group nature and does not indicate the species of plasmodia in human infections. A negative complement fixation test does not rule out malaria and the specificity of a positive must always be established. One technician can, however, do several hundred such tests in one day.

When the results of 6,507 complement fixation reactions and blood films are compared it is found

that the overall sensitivity of the complement fixation reaction was 62 per cent in contrast to 13 per cent from blood film examination. In direct comparisons of 4,007 coincident tests the serological test yielded 6.9 times as many positive reactions as did thick film examinations.

The close relationship of positive complement fixation to present or recent parasitemia is apparent when the serological test is correlated with the incidence of positive blood films and attack rate. The number of positive complement fixation reactions and positive blood films accompanies the increase in number of attacks. The test is therefore an expression of infection which may be existent or recent.

Study of the complement fixation reactions over the 6 months' period shows that the patients fell into serological groups; i.e., the positive pattern group (continued positive), the negative pattern group (continued negative), and the changing pattern group (series of negative reactions alternating with series of positive reactions). Eighty-eight per cent of the patients in the positive pattern group relapsed in contrast to 42 per cent of those exhibiting negative patterns. Seventy-nine per cent of patients with changing patterns relapsed. Series of positive tests at some time were recorded for all of these patients. Thus continued positive complement fixation would appear to offer substantial grounds for prediction of relapse. Further evidence for this statement is furnished when the over-all incidence of positive complement fixation is related to the day of attack. The positive serological test persists in these relapsing patients during the interattack period, but shows a rising curve with attack. The complement fixation blood film ratio on the day of attack was 0.7:1; three days after attack this was 16:1 and 6 days after attack, 80:1.

The persistence of positive complement fixation after the institution of treatment and disappearance of parasites from blood films is the best recommendation for the test.

With eventual elimination of all parasites the serological test becomes negative and remains so. Therefore a series of negative complement fixation reactions accompanied by negative blood films offer good prognosis of cure providing the observations are extended for a sufficiently long time period. Because of individual variation it would be impossible to predict when cure may take place or to determine exactly when all parasites are eliminated. We do not believe that the 6 months' period of this study was long enough to rule out the possibility of relapse. Freedom from relapse is, after all, the only true criterion of clinical cure.

CONCLUSIONS

The complement fixation test for malaria is believed to be worthwhile in study of men returning from malarious areas, who have been under suppressive drug treatment. It may give evidence of persistent vivax infection when blood films are negative and useful information as to the probability of relapse.

A conservative attitude toward the test is recommended: that is, one which recognizes its limitations but appreciates the possibilities of its correct use.

ACKNOWLEDGMENT

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SCRUB-TYPHUS

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The purpose of this communication is to report our experiences with scrub typhus fever in New Guinea. A great deal has been written about scrub typhus during the past year by officers in medical installations of both the Army and the Navy. Some of the discussion has received space in popular magazines as well as in the scientific journals. Because of the location of our hospital, the authors of this paper had the opportunity to observe cases from various endemic foci in the New Guinea Archipelago. Thus it was possible to study the disease in the mildest as well as in the most virulent forms yet to be seen in the New Guinea Archipelago. We feel called upon therefore to emphasize some previously reported features and correct what we have concluded to be certain misconceptions. The study includes 135 patients evacuated into this Hospital from forward areas in the New Guinea Archipelago, together with thirty-eight patients who contracted their disease on this base. Included in the cases whose disease was contracted on this base is a group of sixteen patients from one unit who developed a highly virulent form of the disease in an epidemic fashion over a period of three weeks. More details about this group of patients will be given in subsequent parts of this paper.

Scrub typhus fever is an endemic rickettsial disease having a wide geographical distribution in the Asiatic Pacific area including Japan, Formosa, Korea, Malaya, Ceylon, New Guinea, Pescodores, Philippines, Indo-China, and other islands of the Bismarck Archipelago (1, 4, 5, 6, 8, 9). The ecology, epidemiology, etiology and endemic features of this disease have been carefully reviewed and studied in a recent paper by Kohls et al. (9). The most common vectors in New Guinea are *Trombicula fletcheri*, *Trombicula walchi* and *Trombicula deliensis*. The vector at this base was found to be *Trombicula deliensis*. There seems to be a marked variation in the virulence of the etiologic agent, *Rickettsia orientalis*, in the various areas and this apparently changes from time to time in the same area. The mortality rate has varied in the New Guinea Archipelago from 0.5 per cent to 27.5 per cent (10). The area having the lowest mortality has had the highest incidence of the disease. In this particular area there were eight deaths out of the first one hundred patients hospitalized with scrub typhus, but there have been over 1400 patients subsequently hospitalized from this same area without a single death (10). The mortality rate in various areas of Formosa has been estimated to be as high as 60 per cent. The high incidence of the disease in certain of the endemic areas makes it a serious military problem. The incubation period for scrub typhus has been variously estimated to be from seven to fourteen days. The shortest period found in the New Guinea Archipelago by Kohls et al. was seven days. This was computed from the fact that the first case appeared on the seventh day after a landing on a certain island.

There are several features noteworthy of mention in the small outbreak occurring in a single unit on this base. This has been the most virulent form of the disease occurring in the New Guinea Archipalago, six deaths having occurred out of a total of sixteen cases admitted from a single unit, giving a mortality rate of 37.5 per cent. There had been no deaths reported among cases occurring on this base prior to this time. All of the sixteen cases occurring in the single unit were admitted to the hospital during a three-week period. All gave a history of sitting on the ground in a grassy plot at an outdoor theater. The remainder of the area was very barren of grass or other vegetation. The rat population in the area was very low, and the only mites recovered from trapped rats were identified by Lt. Col. Cornelius B. Phillip as *Trombicula deliensis*. As previously stated in this paper, this has rarely been the responsible vector in the New Guinea Archipelago. There was an abrupt cessation of the outbreak within ten to fourteen days after removal of the outdoor theater to a new area.

CLINICAL MANIFESTATIONS

All the classical signs and symptoms described in previous reports were observed in our cases but,

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as in most other diseases, the particular combination varied with the individual. The most common initial symptoms were headache, oribtal pain, fever and chills. It was the latter two symptoms which usually prompted the soldier to seek medical attention, but after the first few days the most distressing symptom was an extremely severe headache. The incidence of the presenting symptoms is shown in table 1.

The initial diagnosis in sporadic cases coming from an organization in which typhus had not been previously reported, was usually dengue. Malaria was another disease with which typhus was commonly confused during the first few days on the assumption that the density of parasites was not sufficient to provide a positive blood smear. Early diagnosis was not aided by OXK agglutination studies since the titer rarely reached diagnostic levels before the ninth day. Our criteria for diagnosis by agglutination with the OXK was a rising titer. In no case was the diagnosis made on the OXK agglutination alone without a rise in titer to 1:80 or above. It was interesting that one group of cases that we observed, coming from one isolated area, failed to agglutinate the OXK strain of proteus organism. In these cases the diagnosis was based on the classical findings of the disease as described in this paper. Hence, it seems fair to say that there are different strains of *Rickettsia orientalis* manifesting marked variation in virulence, as will be shown in this paper, as well as in its ability to produce antibodies in the blood of patients which will agglutinate OXK strains of the proteus organism.

TABLE 1

Incidence of presenting symptoms in the 173 cases in series

SYMPTOMS	NUMBER	PERCENTAGE
Headache	159	92
Orbital pain	107	62
Malaise	82	47
Chills or chilly sensation	81	47
Backaches	26	15
Constipation	21	12
Diarrhea	16	9
Abdominal pains	14	8
Nausea	14	8
Vomiting	12	7

An eschar was one of the most important features of the disease although it could not be demonstrated in 20 per cent. Characteristically, the eschar first appears as a small sore at the site of attachment of the larval mite. Within a few days there appears in the center of the sore a necrotic slough about 4–6 mm. in diameter with a pink to red areola, the total diameter of the eschar measuring 6–10 mm. The primary lesion is usually single but in a few cases two distinct and separate lesions occurred. Other ecthymatous areas from various insect bites which have been scratched and secondarily infected are sometimes confusing in differentiation from a typical eschar. Rarely one sees areas of lymphangitis proximal to the site of the eschar. However, the lymph nodes draining it are always swollen and tender. In addition, generalized lymphadenitis is very characteristic of the disease and serves in the early stages to differentiate this disease from malaria. The various locations of the primary lesion are given in table 2.

TABLE 2

Locations of the primary lesion or eschar

SYMPTOMS	NUMBER	PERCENTAGE
Ankles and lower legs	31	22.3
Upper legs	26	18.7
Axilla	19	13.7
Abdomen	15	10.8
Scrotum	9	6.4
Back	9	6.4
Buttocks	8	5.7
Lower arms and wrists	7	5.0
Upper arms	6	4.3
Head, neck and face	6	4.3
Feet	2	1.4
Breast	1	0.7

A maculopapular rash, appearing most frequently from the 4th to 7th day and as late as the 10th day, was seen in only 61 (35 per cent) of our cases. The rash was limited to the trunk and extremities in 35 (58 per cent) of the cases, to the trunk alone in 22 (36 per cent) and to the extremities alone in only 4 (6 per cent). The average duration of the eruption was six days. Several patients showed a transitory rash lasting only 1 or 2 days. Special mention should be made of a more diffuse urticarial type of lesion seen in two cases which went on to fatal termination.

Towards the end of the first week, about one third of the patients showed subconjunctival hemorrhages. As the disease progressed into the second week, common findings were: profound

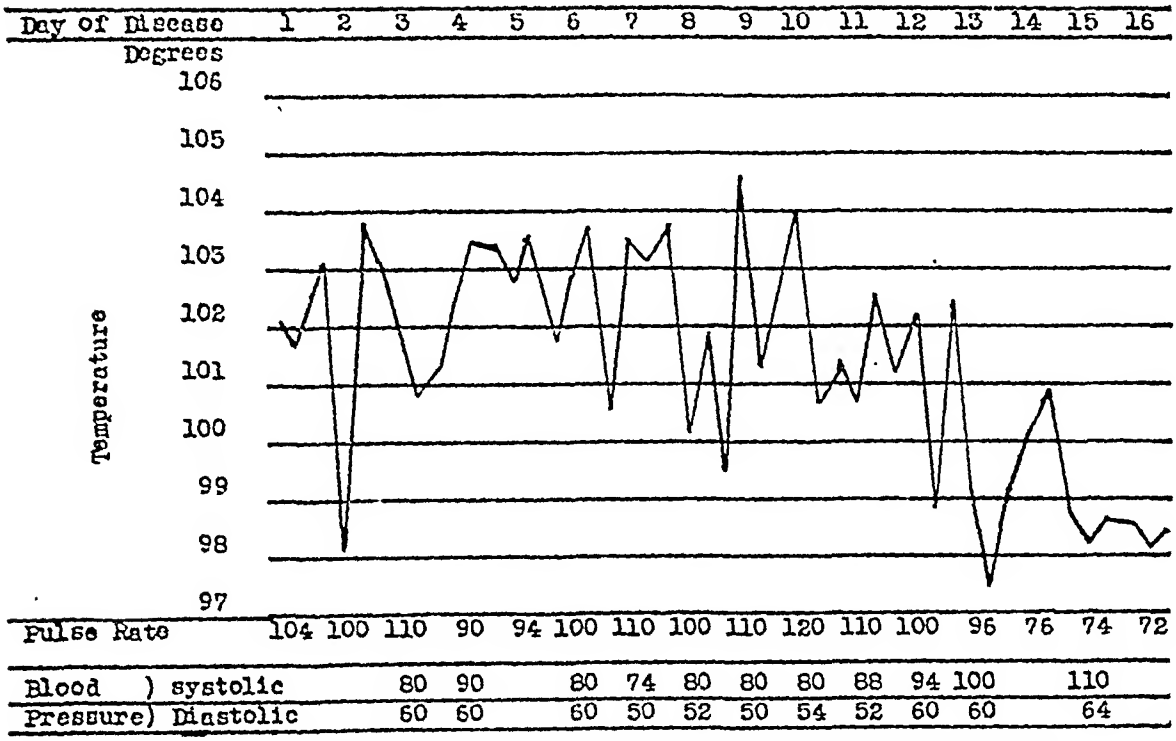

CHART 1. TEMPERATURE, PULSE AND BLOOD PRESSURE GRAPH OF A CASE THAT RECOVERED

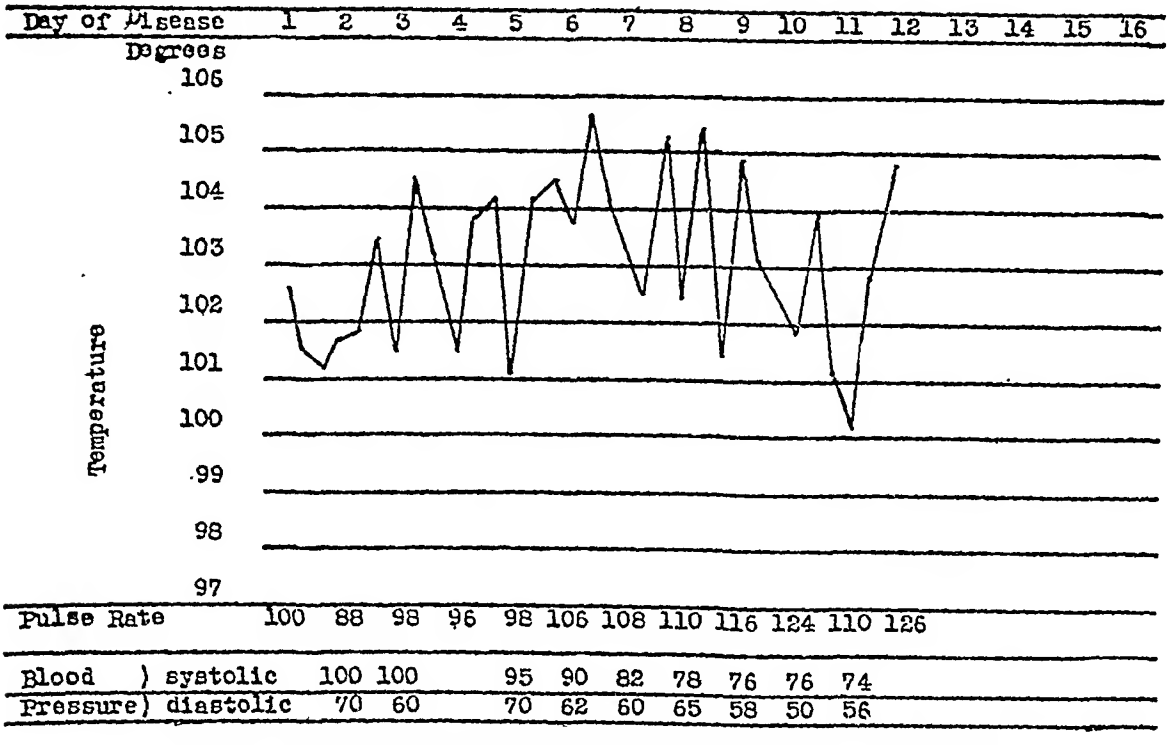

CHART 2. TEMPERATURE, PULSE AND BLOOD PRESSURE GRAPH OF A FATAL CASE

anorexia, intractable headache, nausea, vomiting, constipation, abdominal distention, backache, disturbed sensorium (as apathy, confusion, delirium, insomnia and torpor), conjunctivities, a dusky cyanosis (which could not always be explained by respiratory embarrassment), tachycardia, dyspnea, precordial pain, muscle pains, diaphoresis, tinnitus, hypotension, heart murmurs, cough, and rhonchi in the lungs. Splenomegaly was found in 14 (8 per cent) and hepatomegaly in 11 (6 per cent) of the patients.

The fever was of the remittent type. During the first week, the temperature would rise to increasingly higher levels on successive days (see charts 1 and 2). By the end of the first week, levels between 103 and 105 degrees were often attained. During the second week it maintained a fairly constant daily peak, with defervescence (when it did occur) by lysis. The febrile period varied from 3 to 32 days. There were only 5 (3 per cent) who were febrile less than 14 days. The febrile period lasted from 14 to 21 days in 80 per cent of the cases, and for more shan 21 days in only 17 per cent.

Anemia was not a common finding during the febrile course. However, in a few instances, the red cell count was as low as 2.34 millions. Contrary to common belief, leucopenia was not a frequent finding. The leucocyte count varied from 1,500 to 19,050 per cu. mm. with the majority within normal range. A fairly characteristic feature of the differential blood count was a lymphocytosis ranging as high as 79 per cent and usually above 40 per cent. The lymphocytes were chiefly of the large variety, resembling in many respects the cells found in infectious mononucleosis. However, in all cases in which heterophile antibody titers were done, negative results were obtained. Blood chlorides were not appreciably reduced. Clot retraction and bleeding and clotting times were normal. There was no deviation from the normal in total plasma proteins. Sedimentation rates were elevated in all cases, ranging from 20 mm. to 50 mm. per hour (Wintrobe method). Two cases terminating fatally showed azotemia with accompanying acidosis. Spinal fluid studies with respect to dynamics, cytology, and total proteins were normal.

The clinical and electrocardiographic changes of the cardio-vascular system in scrub typhus are similar to those found in other infections, as typhoid fever, lobar pneumonia and epidemic typhus (7). The early finding of tachycardia is significant, and a rate exceeding 120 per minute usually indicates the presence of a complication. An increase over this arbitrarily set limit indicates a more serious prognosis. It is usually during the second week of the febrile course of the disease that a lowered blood pressure with its accompanying peripheral vascular failure will occur. In addition, in most cases with a cardiac rate of over 120, cyanosis of varying degree and dyspnea may be noted. Just what significance congestive heart failure has in the development of profound vascular collapse, has remained a moot question. We believe the mechanism responsible for the circulatory collapse arises from the effects of damaged capillaries. That is to say, increased capillary permeability due to the toxins of the infecting agents produces a systemic neurovascular reaction. Such a mechanism requires time to develop and may lead to irreversible changes.

Disturbances of the cardiac rhythm, as premature beats, gallop rhythm, and pulsus alternans were occasionally observed. Apical blowing systolic murmurs were frequently heard toward the end of the second week, although no clinical evidence of cardiac dilatation was present to account for the murmur of relative insufficiency. We have not encountered gross arrhythmia, except perhaps as a terminal event in one fatal case.

Electrocardiographic studies during the febrile period of the disease were done in 116 cases, 15 per cent of the electrocardiograms showing abnormal tracings. The abnormalities fell into two groups. The first consisted of changes in the various complexes proper, the most frequent being alterations in the T waves. The earliest changes, usually during the second week, were found to be depression of ST segments and flattening of T waves. As the cases were followed, T waves became progressively lower, isoelectric, diphasic and finally inverted, with or without combination of ST segment depressions. While these T wave changes occurred in all leads, including the chest leads, they were most pronounced in Lead 2. The second group of abnormalities consisted of conduction defects, which were less frequent. We had 2 cases of prolonged P-R interval, 2 cases of intraventricular block with all T waves inverted, and one case of A-V dissociation in the form of complete heart block. Appearance of arrhythmia during the acute stage should always call attention to a conduction defect.

It is obvious from the natural history of the disease, that the acute changes in the cardiac

muscle, although important from the prognostic point of view, will have their real significance in the convalescent state. Briefly, in a period of 7 months, 112 cases were studied with electrocardiograms during convalescence, roughly 1 month after the onset of the illness. Of the total of 112 cases, 16 or 14 per cent were abnormal. The changes again chiefly consisted of low QRS voltage, T wave and ST segment changes with a few cases of conduction defects. This percentage and the findings are in close accordance with observations by others. In accordance with data (12) received from the chief of the cardiovascular section of a numbered general hospital on this base, it was found that out of 123 cases of convalescent scrub typhus 20 per cent showed electrocardiographic abnormalities. This incidence of abnormalities is somewhat higher than ours because only those who did not recover in the usual period of convalescence were transferred to a general hospital. Of the patients showing abnormal electrocardiograms, 40 per cent returned to normal within 1 month. The duration of the heart muscle damage is unknown because we have not had a sufficiently long period of observation of these patients while overseas. However, of 4 patients recently transferred to a general hospital because of myocardial damage, as shown by electrocardiograms, 3 showed normal tracings within 1 month.

The well known symptoms of neurocirculatory asthenia, with its concomitant neuropsychiatric traits in the convalescent state, should be cautiously interpreted in these patients. This is advisable in the light of abnormal electrocardiographic findings, which are an expression of a poor physiological state of the muscle. Therefore all of these patients should receive thorough cardiac study, including electrocardiograms, before being sent back to duty.

Complications observed during the acute phase are listed below in order of frequency in table 3.

The pulmonary complications observed were difficult to interpret both from a clinical and roentgenographic standpoint. They usually have been interpreted as bronchopneumonia or congestive heart failure. However we believe that in view of the post mortem findings in our cases, that neither of these interpretations are tenable.

Clinically, the terminal pictures in our six fatalities were as follows: Three could be classified as respiratory deaths or pulmonary edema, two had convulsive seizures before death, and one sudden death (probably ventricular fibrillation), occurred with no indication that a fatal outcome was imminent.

PATHOLOGY

This discussion will be limited to the pathological findings in the six deaths of this series. All showed lymphadenopathy and evidence of marked weight loss. An eschar was present in all but two patients, who showed all of the other classical pathological features of scrub typhus found in the remaining four cases. The outstanding gross pathological features of this disease are: splenomegaly, hepatomegaly, extreme fluidity of the blood, absence of thromboses, absence of blood clots in all vessels, and evidence of a diffuse hemorrhagic diathesis. The latter was most marked in the lungs, heart, spleen and gastro-intestinal tract.

In general, the microscopic lesions common to all organs were: swelling and desquamation of the endothelium of capillaries, edema, marked diffuse interstitial infiltration with inflammatory cells (lymphocytes, plasma cells, monocytes, histiocytes, and occasionally polymorphonuclear leucocytes), and parenchymatous cellular degeneration. Spotty perivasculitis was found in all cases. Petechial hemorrhages were common in the pleurae, epicardium, spleen, lymph nodes, testicles, serosa and mucosa of bowel and pelvic mucosa of the kidneys. Another common finding was the presence in the lymph nodes, and to a lesser extent in the spleen, of multinuclear giant cells having an eosinophilic and fairly scant cytoplasm.

TABLE 3

Complications in the acute phase of scrub typhus

COMPLICATIONS	PERCENTAGE	COMPLICATIONS	PERCENTAGE
Impaired hearing	20	Retinal hemorrhage	2
Pulmonary complications	17	Hepatitis	1
Abdominal distension	10	Malaria	1
Arthralgia	4	Herpes zoster	1
Epistaxis	5	Hiccough	1
		Encephalitis	1

The heart revealed, in all cases, myocardial edema and epicardial inflammatory cell infiltration. Most cases had cellular infiltration of the myocardium, and half of the cases showed it in the endocardium. The infiltration was predominantly diffuse and only occasionally perivascular. The

cell types were largely those of chronic inflammation. A large number of polymorphonuclear leucocytes was found in only one case where the infiltration was particularly impressive. This case also had edema of the entire heart, extensive petechiae and a hydropericardium. In 5 cases the vasa vasorum of the aorta, or the intima of the aorta, displayed round cell infiltration. Epicardial petechiae were found in 4 cases, and in 3, swelling of the capillary endothelium. Three showed a pericardial exudate and cloudy swelling of the myocardium. In one case the myocardium was exceedingly flabby; this case also showed marked epicardial hemorrhages and bloody pericardial fluid. The average heart weight was 368 grams and varied between 260 and 480 grams.

The respiratory system showed diffuse or focal consolidation of the lungs in all except one of the cases. The cut surfaces of the lungs were gelatinous and the fluid expressed was bloody and slightly foamy. The alveolar septa were thickened due to edema, congestion and inflammatory cell infiltration in all cases. There was swelling and desquamation of the capillary endothelium and the alveolar epithelium with extensive capillary hemorrhages, old and recent. This hemorrhage into the alveoli and interalveolar septa was a very striking feature. The bronchial exudate was viscid, due to the high number of cells, inflammatory, phagocytic and epithelial. Two cases also showed degeneration and desquamation of the bronchial mucosal epithelium. The pleural tissue showed inflammatory cell infiltration. One case showed multiple pulmonary abscesses, together with a fibrino-purulent hemorrhagic pleural exudate. One case displayed a small active tuberculous lesion. In two others, the bronchial lymph nodes showed areas of caseation and calcification. The average weight for the right lung was 749 grams, for the left lung 714 grams and varied from 350 to 1095 grams.

The changes in the spleen and hemopoietic system were rather constant. There was definite splenomegaly in all cases. The average weight of all spleens was 637 grams and varied from 360 to 1210 grams. The large spleens were soft, friable, and had bulging cut surfaces from which the pulp was scraped off with ease. The largest spleen showed a solitary abscess. The smallest spleen, which weighed 360 grams, showed multiple infarcts with no evidence of thrombosed vessels to account for them. This was the case with the pulmonary abscesses. All spleens showed congestion, old and recent hemorrhages, infiltration with plasma cells and polymorphonuclear leucocytes. The spleens also showed marked swelling and desquamation of the sinusoidal endothelium.

All lymph nodes were edematous, congested, and had only small follicles. All showed desquamation of the sinusoidal endothelium, and infiltration with plasma cells and polymorphonuclear leucocytes in most of them. In all nodes a few multinucleated giant cells were found having an acidophilic scanty cytoplasm with 2–6 large round nuclei, very similar to Dorothy Reed cells. One showed a focal necrosis, another a recent hemorrhage.

The gastro-intestinal system in all cases revealed congestion and edema of all layers and a mild diffuse round cell infiltration. One case showed a considerable peritoneal exudate and peritoneal petechiae. This case also had a fresh gastric ulcer.

The liver showed a distinct enlargement in all cases. The average weight of all livers was 2387 grams. The largest weighed 3330 grams. There was edema in all of them and marked inflammatory cell infiltration of the portal spaces, which in most cases also extended between the liver cords and into the capsule. This infiltration was distinctly not perivascular; no cuffing was found. Most livers showed cloudy swelling and mild central fatty vacuolation. Some desquamation of capillary endothelium was occasionally noted.

The pancreas in most cases was moderately enlarged up to 230 grams and showed some edema with mild round cell infiltration into the septa. In only one case was there a diffuse interstitial pancreatitis.

The genito-urinary system revealed edema and cloudy swelling in all kidneys. Four showed some desquamation of the glomerular capillary endothelium; in two cases the tubules were distinctly flattened. In all of them there was a diffuse inflammatory cell infiltration, while cuffing was noted in only three of them, and in the latter only around capillaries which were filled with inflammatory cells. In four cases the pelvic mucosa showed petechial hemorrhages; in another there was considerable perirenal hemorrhage. The average weight of the kidney was 263 grams and varied from 190 to 330 grams. The bladder was similar histologically to the gastro-intestinal tract.

In two cases the prostate showed a diffuse cellular infiltration with slight cuffing of some of the capillaries.

The gross appearance of the adrenals was nor-

mal. The outstanding microscopic features were congestion, edema and mild round celli nfiltration. In the case who died on the 23rd day of his illness there were areas in the cortex of foamy and disintegrated cells whose nuclei were fairly well preserved.

All testicles were edematous and showed a diffuse cellular infiltration with only occasional cuffing. The same was found in the sections of the epididymides. Petechiae were found in one testicle; in another case they were located in the tunica propria of this organ.

In the central nervous system, the lesions were not only pronounced, but surprisingly uniform. Meningeal edema, with diffuse inflammatory cell infiltration, was very pronounced. Swelling of capillary endothelium in the leptomeninges, with accumulation of inflammatory cells within the lumina, and perivascular cuffing was quite characteristic. There was cerebral edema in all cases and in one it was very marked, the brain weighing 1980 grams. Early degenerative changes were were noted in the ganglion cells of all cases, but these changes were never very distinct. There was moderate gliosis in all cases, both in cortex and white matter. In some cases this gliosis was diffuse, in others it was frequently most pronounced around degenerating capillaries and associated with collections of lymphocytes and other round cells. The pituitary showed edema but no cellular infiltration.

The most outstanding pathological features in each of the six fatal cases are shown in the following paragraphs.

Case 1 was 27 years of age and died on the 14th day of his illness. The endothelial changes of the coronary vessels and the vasa vasorum of the aorta were quite pronounced. The pulmonary hemorrhages were extensive. The inflammatory changes of the pleura were marked. The spleen was large (660 grams), and the swelling and desquamation of the sinusoidal endothelium was pronounced. The pancreas showed interstitial inflammatory changes. The agglutination with the OXK at autopsy was 1:20. His terminal clinical picture was that of pulmonary edema.

Case 2 was 26 years of age and died on the 11th day of his illness. The heart was unusually flabby, covered with petechiae, while the pericardium contained 20 cc. of hemorrhagic fluid. There were also perirenal hemorrhages, and petechiae in the testicles. There were only mild pulmonary changes and evidence of only recent hemorrhage. The pleurae also showed petechiae. The agglutination with the OXK at autopsy was 1:160. There was no eschar. This is the patient whose terminal clinical picture included severe convulsive episodes. The brain showed the most extensive changes, being 50 per cent heavier than normal and both grossly and microscopically showed very extensive edema.

Case 3 was 30 years of age and died on the 14th day of his illness. He had the largest spleen (1210 grams) and the largest liver (3330 grams). He also had the largest heart (480 grams). There was marked edema of the heart and the liver, together with marked inflammatory cell infiltration. The spleen had a solitary infarct. Another solitary infarct was found in the right kidney. There was a slightly active small tuberculous lesion in one lung. The agglutination with OXK at autopsy was 1:320. It was this patient who had a sudden unexplained death on the 14th day of his illness, with no indication that death was imminent. The interpretation at the time was that ventricular fibrillation had occurred to precipitate his death.

Case 4 was 30 years of age and died on the 12th day of his illness. There were petchiae of the epicardium, of the pleura and of the pelvic mucosa of the kidneys. The pericardium contained 10 cc. of cloudy yellowish fluid. The lungs showed extreme edema and hemorrhage. There was a caseous and calcific bronchial lymph node. Another lymph node showed a recent hemorrhage. The agglutination with the OXK at autopsy was 1:160. His terminal clinical picture was that of pulmonary edema.

Case 5 was 36 years of age and he died on the 23rd day of his illness. The heart showed marked edema of all portions, and the most acute inflammatory cell infiltrations of all the cases. There were petechiae of the epicardium, pleurae, peritoneum and the tunica vaginalis of the testicles. The pericardial sac contained 150 cc. of clear fluid and the peritoneal cavity 500 cc. of clear fluid. The gastric mucosa showed a fresh ulcer. This was the only case with bronchopneumonia in addition to the extensive pulmonary hemorrhage and edema. The agglutination with OXK at autopsy was 1:40. It was thought that this patient died from sheer exhaustion because his illness had such a severe and prolonged course.

Case 6 was 33 years of age and died on the 14th day of his illness. His case differs most from the pattern set by the other five cases. His heart

was the smallest of this series (260 grams) and showed only moderate inflammatory changes. The lungs showed multiple abscesses in addition to extensive pulmonary hemorrhage and edema. There were 200 cc. of cloudy brownish fluid in the right pleural cavity and fibrin deposits over both lungs. The spleen was also the smallest in this series and showed multiple infarcts. There was no eschar. An OXK agglutination was not performed at autopsy. A biopsy of the skin, however, showed endothelial swelling and perivascular cuffing of inflammatory cells. His terminal picture was that of pulmonary edema.

The average age at death was 30.3 years. The youngest was 26 and the oldest 36. Among the sixteen cases occurring in one unit, the average age of the ten survivors was 23.9 years. The youngest was 21 and the oldest 34. This would seem to be a significant difference in age, indicating that the younger age group tolerates the disease better.

The average duration of the disease before death in these six cases was 14.6 days. The shortest duration was eleven days. The only case surviving over 14 days died on the 23rd day of his illness. It may be deduced from these data that if a patient is under 25 years of age and survives the disease for more than 14 days, that he will probably recover.

The explanation for the presence of the multinucleated giant cells in the lymph nodes and spleen is not known. There has been no reference to these cells in the literature on scrub typhus which we have reviewed. The extreme fluidity of the blood could not be explained; however, it could be due to a prothrombin deficiency. The hemorrhagic diathesis in those cases is similar to, if not identical with, that seen post mortem in cases dying of acute infectious hepatitis, all of which have a marked prolongation of the prothrombin time. Unfortunately only one of the fatal cases had a determination of the prothrombin time, bleeding time, clotting time, platelet count and clot retraction time. All of these tests were within normal limits three days before death. However, many of the other serious but non-fatal cases, with or without hemorrhagic diasthesis, showed normal values for the above tests.

TREATMENT

It should be emphasized that the severity and mortality of the disease in any one area is dependent on the particular virulence of the rickettsial strain in that area. We feel that this concept has not been thoroughly emphasized in the literature coming to our attention.

In the absence of specific therapy, the entire aim of the treatment is to maintain the strength of the patient over a period of time sufficient for the production of enough antibodies to overcome the infection. Absolute bed rest is the most important factor. Thus, it is advocated that a special nurse be provided for each severe case and that the patient be permitted to do nothing for himself. The nurse is indispensable for frequent determinations of blood pressure during the critical periods of the illness.

In our group of cases oxygen was used very early in the disease at the first sign of increased respiratory rate with or without cyanosis. Although our experience may be at variance with others, we have found that the use of an oxygen face mask only served to increase the apprehension of the patient. The oxygen tent proved to be the most ideal means of administering oxygen. The tent had the further advantage, during the period of hyperpyrexia, of being air conditioned.

An effort was made to secure an adequate fluid intake of at least 3000 cc. daily by the oral route. Yet, to maintain electrolyte balance, in many cases a daily hypodermoclysis of 1000 cc. of normal saline was indicated. Intravenous administration of fluids was avoided as much as possible, because it was felt that too frequent use of this route might overtax a poorly functioning circulatory system. Blood plasma was used to treat what we interpreted as medical shock, in which the systolic pressure fell below a critical level of 70 mm. of mercury and the pulse rate above 120 per minute; however we were not impressed with its efficacy. Many cases showing the same symptoms did as well or better without the use of intravenous plasma. The use of epinephrine and cortin in these shocked cases proved to be of no benefit in the few patients in whom it was tried. If one considers the disturbances in the physiology, this is what one would expect. These shocked cases seemed to improve most satisfactorily by using only sedation and good nursing care.

Morphine was avoided in the presence of respiratory embarrassment in the belief that this drug would depress respirations. A second consideration motivating against the use of morphine was its tendency to increase the very distressing symptom of abdominal distention. The latter was best treated with colon tubes and pitressin or

prostigmin methyl sulfate. The use of analgesics such as codeine, aspirin, or a combination of the two drugs, were at times helpful. In severe mania and convulsions, intravenous barbiturates had to be used occasionally; however, most cases of agitation, delirium and insomnia could be controlled with oral doses of barbiturates and chloral hydrate. Hiccuping, also a troublesome complication, was best controlled by gastric lavage.

Citrated whole blood (500 cc.) from a patient convalescing from scrub typhus was used in one case and intramuscular injection of the patients own blood was used in 3 cases, with no apparent benefit from either. Spinal puncture with withdrawal of 10 to 30 cc. of fluid failed to afford relief from headache in most cases, while in others the relief, if any, was only temporary. Sulfa drugs and penicillin were not used in any of our cases because they are known to be ineffective against the Rickettsia orientalis, and we encountered no bacterial complications for which they would be indicated. We found no indication for digitalis because there were no cases showing classical signs of congestive heart failure, and because the use of this drug in the presence of an acute myocarditis is open to question. Co-existent malaria was treated with atabrine.

With regard to prophylaxis, Kohl et al., state that "without specific means of immunization, control measures must be dependent on the manipulation of environmental factors, combined with personal protection according to present Army Regulations."

CONVALESCENCE

In cases coming to our attention, convalescence for at least two months was required because of weight loss from 8 to 53 lbs. (average of 23 lbs.), persistent headache, weakness, easy fatiguability, tachycardia, palpitation, dyspnea on exertion, minor residual electrocardiographic abnormalities, persistent arthralgia, residual deafness, residual defective vision and persistence of an elevated sedimentation rate. Even at the end of this period, some patients had to be reclassified to a noncombat status or transferred to general hospitals. It is conceivable however that in less virulent outbreaks, a shorter period of convalescence would suffice.

Following defervescence, the patients remained at absolute bed rest for at least three weeks, and a great many required a longer period. After six weeks of fairly close bed rest, limited activities were prescribed. All typhus patients at the same stage of convalescence were grouped together in one ward, so that their activities could be more efficiently managed. A gradually increasing program of daily activities, commensurate with their physical capacity, was carried out under the close supervision of a medical officer. This included boat rides, picnics, calisthenics, and more strenuous sports as their condition improved. This program was designed not only to speed convalescence but also to provide a fairly accurate test of the amount of activity a patient could tolerate. It was felt that only with this information at hand could a medical officer make a fair estimation of the type of duty for which the individual was best suited.

TABLE 4

Complications requiring transfer to a general hospital

COMPLICATIONS	NUMBER OF CASES	COMPLICATIONS	NUMBER OF CASES
Psychoneurosis	7	Headaches and vertigo	1
Neurocirculatory asthenia	6	Hepatitis	1
Myocardial damage	4	Malnutrition	1
Optic neuritis	1	Malaria	2
Headache	1	Psychosis	1
Headache and nystagmus	1	Duodenal ulcer	1
		Total	27

Before discharge to duty, or transfer to a general hospital for further observation and disposition, complete physical examinations, electrocardiographic studies, and sedimentation rates were done on all patients, so that a careful final estimation of his physical condition could be ascertained. No patient was returned to duty in whom any marked abnormality was found. Patients showing evidence of psychosis, psychoneurosis, persistence of an abnormal electrocardiogram suggestive of myocardial damage, or manifestations of vasomotor instability such as tachycardia, palpitation and transient hypertension or other complications, were transferred to a general hospital.

Some of the complications in convalescence requiring transfer to a general hospital are shown in table 4.

COMMENTS

There were no deaths in the group of 135 patients admitted to this hospital from forward areas. However, it was necessary to transfer 20 of them to a general hospital because of various complications shown in table 4. There were 6 deaths in the 38 patients who contracted their disease on this base, and all the deaths occurred in one unit having 16 cases over a period of 3 weeks. Hence the mortality for this particular unit was 37.5 per cent, which is the highest mortality thus far reported in this theater and serves to emphasize the marked variability in the virulence of the various outbreaks in the same as well as in different localities in the New Guinea Archipelago. Another index of the virulence of the outbreak in the unit having the six deaths is that five of the ten that survived had to be evacuated to general hospitals because of persistent headaches in 3, psychosis in 1, and neurocirculatory asthenia in 1.

The frequency of psychoneurosis deserves further comment. Patients who recovered had a further obstacle to surmount, namely, the belief obtained from articles in popular magazines that the disease rendered the individual a cardiac invalid for the rest of his life. For this reason, or for other similar reasons, a small percentage manifested an anxiety state which extended well into convalescence and which was characterized by palpitation, tremulousness and, in significant number, a feeling of constriction in the chest. This group of symptoms we have called neurocirculatory asthenia because we felt it a more descriptive term, in spite of the fact that more recently these symptoms have been classified as an anxiety type of psychoneurosis.

In the over-all picture of the 173 cases observed by us, 140 (81 per cent) were returned to full duty after an average hospital stay of 8 weeks. The shortest period of hospitalization was two weeks and the longest sixteen weeks. Twenty-seven patients (15.5 per cent) were transferred to general hospitals for further observation and disposition.

It is our belief that 75 per cent of the cases transferred to general hospitals will be returned to duty within a period of 3 months, and that the remaining 25 per cent will be returned to duty within 6 months. There is possibly one exception to this statement, which is the patient with what was at first thought to be a toxic or postencephalitic type of psychosis. However, subsequent study revealed that he had a fairly typical scizophrenic type of psychosis which might well have developed at the time without any illness. In view of the more recent diagnosis, it hardly seems fair to say that this is a sequelae of scrub typhus fever. Although we have no positive proof, it is our feeling that there are no permanent sequelae of this disease.

The disturbance in physiology in the patients having a very virulent form of the disease warrants further comment. There were few or no alterations in the cellular, protein or chemical constituents of the blood, spinal fluid, or urine in any of the patients except in the terminal phases of those having a fatal outcome. These studies included repeated blood counts, bleeding and clotting time, hemoglobin determinations, and urinalyses. In the more seriously ill group, many had roentgenograms of the chest, spinal fluid analyses and determinations of platelet counts, clot reaction time, prothrombin time, serum bilirubin, total and fractionated plasma proteins, non protein nitrogen or blood urea, blood chlorides and CO_2 combining power.

The chief disturbances in physiology in the more serious and fatal cases were changes resulting in an acidosis, azotemia, proteinuria, depressed respiration, and changes in the cardiovascular system. The acidosis is most likely due to two factors: the first is the result of the marked changes in the lungs with a decreased ability to excrete CO_2, giving an uncompensated excess of CO_2; the second is the result of the profound changes in the liver resulting in decreased glycogen storage and ketosis so that the excess of ketones are excreted in the urine carrying with it $NaHCO_3$, giving an uncompensated alkali deficit. The azotemia is very suggestive of the extrarenal type in view of the minor pathologic changes found in he kidneys (11). The shallow and finally apneic respirations are probably brought about by a number of factors including general anoxia, Hering-Breuer reflex, and by ischemia or local anoxia of of the respiratory center itself through local capillary damage. The histological changes in the cardiovascular, and particularly the capillary system, are the most outstanding and it is the effect of these changes that accounts for the death of patients. As shown in the pathological examination, edema of all tissues was very marked and in the lungs more pronounced than in any other organ. In addition to the fact that the lungs were the most edematous organs, there was also an outpouring of all the cellular elements of the blood into the lungs. This change is undoubtedly due to

local capillary damage with increased permeability. Following this extravasation in the lungs, anoxia results because of decreased aeration of the blood. This then causes a vicious cycle because of the damaging effect of the anoxia on the capillaries. The changes in the heart itself were not as marked as has been reported by other observers. One death could perhaps be directly attributable to cardiac damage with sudden death from ventricular fibrillation. One of the other five deaths, having a convulsive seizure as a terminal event, was most likely due to cerebral edema as shown in the pathology report. The other four deaths are perhaps best explained on a pulmonary basis, being directly attributable to suffocation resulting from extravasation of all the elements of the blood through the capillaries in the lungs. The increase in capillary permeability is more manifest in the lungs because of the decrease in supporting structure around the capillaries in these organs as compared with other organs of the body, together with the added trauma of respiration. It is very difficult, clinically or by roentgenograms, to differentiate this picture from bronchopneumonia or from congestive heart failure except that none of our cases showed the classical signs of venous engorgement found in congestive heart failure. This probably accounts for the reports in the literature of a high incidence of congestive heart failure and bronchopneumonia. The latter was found in only one case at post mortem. It is from an understanding of the disturbed physiology and histology in these fatal cases that one would not expect any response to treatment of any sort without control of the factors causing the increased capillary permeability. Until a means of controlling the infecting agent itself is found, there seems little to offer in the way of specific treatment for these patients critically ill with scrub typhus fever.

SUMMARY

1. The classical signs of the disease are an eschar, lymphadenitis, headache, orbital pain, remittent fever up to 105° for fourteen or more days and a maculopapular rash. The convalescent period usually lasts from 6–8 weeks.

2. The diagnosis is made on the basis of the classical signs and a rise in titer up to 1:80 or above in the agglutination of the OXK strain of the proteus bacillus.

3. The outstanding features observed in a series of 173 cases of scrub typhus fever occurring in the New Guinea Archipelago during the last 8 months of 1944 are reported.

4. The mortality in the entire group was 3.5 per cent: however, all six deaths occurred in a single unit at one locality, having a total of 16 cases during a 3 weeks period. It should be emphasized that the mortality rate depends on the virulence of the Rickettsia orientalis at a given time in a particular area and that this may change rom time to time in the same, as well as in different localities.

5. The disturbances in physiology in the serious and fatal cases, and the pathology found in the six fatal cases, have been presented and discussed. The transitory nature of the changes in the cardiovascular system of the cases that recovered have been emphasized. The common error of interpreting the pulmonary complications due to extravasation of blood into the lungs as bronchopneumonia or congestive heart failure have been emphasized.

6. Eighty-one per cent of the entire group were returned to duty within an average period of 8 weeks. It was necessary to prolong hospitalization in 15.5 per cent because of certain temporary sequelae listed in this paper and other coexistent complications.

7. The program of management during the acute and convalescent phases of the disease have been outlined and discussed.

8. The complications and sequelae have been enumerated in order of frequency. We are of the opinion that there will be no permanent sequelae of this disease.

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OBSERVATIONS ON THE INCIDENCE OF WUCHERERIA BANCROFTI LARVAE IN THE NATIVE POPULATION OF THE SOLOMON ISLANDS AREA[1]

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In making a routine malaria survey on Solomon Island natives, microfilariae of *Wuchereria bancrofti* were encountered in addition to the malaria organisms. The demonstration of the filaria parasite in the blood stream of this native group seemed to justify further study to ascertain the incidence of infection among these natives. The present paper gives the results of this study.

A total of one thousand, two hundred and sixty-three (1263) individuals from Guadalcanal, Malaita and San Cristobal of the Solomon Islands, one hundred forty-six (146) Fiji Islanders and three hundred thirty-two natives from the Gilbert Islands were surveyed specifically for the purpose of demonstrating the presence of filaria larvae in the blood stream. Throughout this survey the thick blood film (as for malaria) was employed. The results of this study are given in table 1.

When the data in table 1 are broken down into incidence data for the various islands from which these natives came, it is seen that those individuals from the island of San Cristobal of the Solomon Islands give the highest filaria rate of any island group surveyed (table 2).

In making the survey it was not possible for all blood films to be taken at a predetermined time. Thus many of the smears were taken during the daylight hours while others were taken after complete darkness had set in. Table 3 gives the results of our findings on smears taken only during the daylight hours as contrasted with findings on smears taken only after nightfall.

The data presented in table 3 strongly suggest that the filaria encountered in the blood stream of those natives included in this survey exhibit a marked nocturnal periodicity of microfilariae. To further demonstrate this indicated periodicity, two tests were undertaken. The first of these dealt simply with taking blood samples (thick films) on the same group of individuals both before and after complete darkness. Table 4 gives the results obtained from this test.

The second test devised to further demonstrate nocturnal periodicity of microfilariae consisted of taking thick blood smears at approximately two hour intervals for a period of twenty-four hours. Three natives previously shown to harbor filaria larvae were selected. The results of this test are given in table 5.

Recently it has been our good fortune to continue the study on the incidence of filariasis in this area by working in close collaboration with the Navy Filaria Survey Unit. Since the arrival of this unit a total of seven hundred thirty-four (734) natives from the Solomon Islands and three hundred thirty-two (332) natives from the Gilbert Islands have been examined by us in conjunction with the Navy group. We have held to the routine malaria thick smear method while the Navy unit employed a measured volume of blood in determining the incidence of filaria infection. In all cases the smears, both thick smear and measured volume sample, were taken at the same time from the same individual and each set of samples was treated independently by the group responsible for the method used. Table 6 shows the independent results of this study together with the indicated incidence as determined by both methods in combination.

It will be noted that the total incidence as determined by both methods is higher than that determined by either method alone. It thus becomes obvious that certain infections were picked up by one or the other method which was missed by the one method alone.

A survey was made of a selected group of natives from the Gilbert Islands for the purpose of determining whether or not the strain of filaria larvae found in these natives exhibit a nocturnal periodicity. Twenty-seven (27) individuals were selected from the fifty-four (54) showing microfilariae in night blood. During daylight hours blood smears were made on this group and the number of microfilariae determined. Table 7 gives the results of this survey. Although a noc-

[1] The author is especially indebted to Lieutenant Elon E. Byrd, H-V(S), USNR for cooperation in making the final part of this survey and for invaluable aid in the preparation of this paper.

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turnal periodicity is strongly indicated, this phenomenon appears to be less clearly defined than is the case noted for the larvae encountered among the Solomon Island natives. The sharp increase in the number of microfilariae circulating in the peripheral blood in the evening is not demonstrated among the Gilbert Island natives as it is among those natives from the Solomon Islands. A comparison of the incidence of filariasis between these two groups is shown in table 8.

One Gilbert Island native was selected for experimental infection of *A. punctulatus punctulatus* and *A. punctulatus farauti*. Both species of mosquito fed on the native at 1930, at which time a 20 cm sample of blood proved to carry fifty-five (55) microfilariae. Dissection of these fed mosquitoes

TABLE 1

Incidence of filaria larvae in natives surveyed from the Solomons, Fiji and Gilbert Islands

NUMBER SURVEYED	NUMBER POSITIVE	PER CENT INFECTION
1,741	331	19.

TABLE 2

The determined incidence of filaria larvae in the blood stream of natives surveyed from the various islands

ISLAND	NUMBER SURVEYED	NUMBER POSITIVE	PER CENT INFECTION
Guadalcanal	157	16	10.2
Malaita	548	56	10.2
San Cristobal	558	176	31.5
Fiji	146	29	19.8
Gilbert	332	54	16.3
Total	1,741	331	19.

TABLE 3

Incidence of filaria larvae determined from "day" blood contrasted to the incidence obtained from "night" blood—Solomon Island Natives

NUMBER SURVEYED	TIME OF SURVEY	NUMBER POSITIVE	PER CENT INFECTION
94	Day	5	5.4
880	Night	180	20.

TABLE 4

Incidence data obtained on the same group of natives when smears were taken during daylight hours and again after nightfall—Solomon Island Natives

HOUR	NUMBER SURVEYED	NUMBER POSITIVE	PER CENT INFECTION
1600–1800	435	18	4.1
2130–2400	435	92	21.1

TABLE 5

Number of microfilariae per thick blood smear sample taken from each of the three natives at the indicated hours

HOUR	NUMBER OF MICROFILARIAE		
	Case 1	Case 2	Case 3
0090–0930	1	0	1
1100–1130	0	0	8
1300–1330	1	1	15
1500–1530	3	4	2
1730–1800	7	15	28
1930–2000	70	93	99
2130–2200	217	42	142
2330–2400	163	108	22
0130–0200	239	97	171
0530–0600	64	56	50

TABLE 6

Incidence of filariasis as determined by the malaria thick smear and the measured volume

ISLAND	TYPE OF SMEAR	HOUR	NUMBER SURVEYED	NUMBER POSITIVE	PER CENT INFECTION
Solomon	Thick	1930–2200	734	128	17.4
Solomon	Meas. vol.	1930–2200	734	142	19.3
Solomon	Both methods	1930–2200	734	151	20.6
Gilbert	Thick	1930–2230	332	52	15.7
Gilbert	Meas. vol.	1930–2230	332	50	15.0
Gilbert	Both methods	1930–2230	332	54	16.3

showed that both species picked up the filaria larvae. However, the microfilariae failed to survive longer than thirty-six (36) hours within *A.*

TABLE 7

Incidence of filaria infection found in Gilbert Island natives during daylight hours as compared to night blood

TYPE OF SMEAR	HOUR	NUMBER SURVEYED	NUMBER POSITIVE	PER CENT INFECTION
Thick smear	1400–1500	27	17	63.
Thick smear.....	1930–2230	27	27	100.
Meas. volume.....	1400–1500	27	14	51.8
Meas. volume....	1930–2230	27	27	100.
Both methods.....	1400–1500	27	20	74.
Both methods.....	1930–2230	27	27	100.

Only a small colony of *A. punctulatus farauti* was available at the time and did not permit compiling any conclusive data with reference to the complete development of the larvae within this species. It has been demonstrated by Byrd and St. Amant that *A. punctulatus farauti* will readily develop to infectivity the larvae of that strain of the filaria parasite found in the Solomon Island natives. Table 9 shows the results of the feeding experiment with the Gilbert Island strain of filaria.

These dissection studies are preliminary and are being continued to establish the degree of efficiency of various mosquitoes as vectors of filaria organisms from different islands. Also an effort is being made to determine any biological difference in the filaria organisms from these different islands.

TABLE 8

Comparison of incidence of filariasis during daylight and evening, between Solomon Island and Gilbert Island Natives

ISLAND	HOUR	NUMBER EXAMINED	NUMBER POSITIVE	PER CENT INFECTION	TOTAL NUMBER OF MICROFILARIAE IN ALL POSITIVES	PER CENT MICROFILARIAE IN DAY BLOOD COMPARED TO NIGHT BLOOD
Gilbert.....	1330	27	20	74	144	11.3
Gilbert.....	1930	27	27	100	1,280	100.0
Solomon.....	1330	95	18	19	*	
Solomon.....	1930	95	95	100		

* Although no record was kept on the total number of microfilariae encountered in this group of natives, such figures are known for the three individuals who were studied at two hour intervals for twenty-four hours (table 5). These figures show that from 0600 to 1800 a total of 86 (5.3%) microfilariae were counted, while 1633 microfilariae were found between the hours of 1800–0600.

TABLE 9

Infection of A. punctulatus punctulatus and A. punctulatus farauti with Gilbert Island strain of Wuchereria bancrofti

	NUMBER FED	NUMBER THAT PICKED UP LARVAE	PER CENT THAT PICKED UP LARVAE
A. p. punctulatus.....	238	17	7.14
A. p. farauti...	24	4	16.66

punctulatus punctulatus, although a single specimen of *A. punctulatus farauti* developed the larvae through the fourth day of larval development.

From the data presented in this paper the following summarized points might be stressed:

Microfilariae of *Wuchereria bancrofti* are demonstrated to be present in the peripheral blood of approximately one-fifth of the local native population thus far surveyed from the Solomon Islands group. Those natives surveyed came from three of the Solomon Islands, Guadalcanal, Malaita and San Cristobal.

Microfilariae of *Wuchereria bancrofti* showed a marked nocturnal periodicity within this area. The larvae reach the peak of their numbers in the peripheral blood between the hours of 2100 and 0200.

A CONCENTRATION METHOD FOR DEMONSTRATING MICROFILARIAE IN BLOOD

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The increasing importance of the filarial diseases and the difficulties in their early clinical diagnosis have created a need for a simple rapid method of demonstrating microfilariae when they are present in the blood stream in small numbers.

Using the microfilariae of *Dirofilaria immitis* (the dog heart worm), we have examined the various methods of microscopic diagnosis which have been described in the literature (table 1). Because no method seemed at once simple, rapid, capable of giving quantitative concentration of larger amounts of blood and also adapted to a non-fatiguing examination of a large number of samples, the following method was evolved.

TABLE 1

METHOD	ADVANTAGES	DISADVANTAGES
Examination of		
1. Fresh thick wet drop of blood	Motile microfilariae	Microfilariae obscured by red blood cells. No concentration
2. Serum expressed from clotted blood	Motile microfilariae	Poor to negligible concentration
3. White cell layer of centrifuged citrated blood	Motile microfilariae	Concentration poor and variable
4. Centrifuged sediment of 1 ml. blood hemolyzed by addition of 5 ml. water*	Excellent concentration, motile microfilariae	Microfilariae are only sluggishly motile. Concentration difficult to achieve with large volumes of blood
5. Sediment of mixture of 1 ml. blood plus 10 ml. 2% formalin allowed to stand for 12–24 hours†	Simple. Centrifuge unnecessary	Variable concentration. Microfilariae immotile. Time consuming
6. Centrifuged sediment of mixture of 1 ml. blood plus 10 ml. 2% acetic acid‡	Excellent concentration	Microfilariae not motile and difficult to see. Method not feasible for large quantities of blood
7. Giemsa stained thick blood film	Excellent definition of structure	No concentration. Microfilariae not motile

* The Animal Parasites of Man. Fantham, H. B., Stephens, J. W. W., and Theobald, F. V. New York: William Wood and Company, 1920.

† Knott, J.: Proc. Roy. Soc. Trop. Med. and Hyg., 33: 191, 1939 (July).

‡ Human Parasitology. Rivas, D. Philadelphia and London: W. B. Saunders Company, 1920.

Four ml. venous blood are drawn and ejected into a tube containing 0.01 ml. heparin solution (Liquaemin, Roche-Organon Inc. 1 ml. contains 10 mgm. purified heparin). Four ml. 2 per cent saponin in distilled water are added and the contents of the tube are gently mixed until hemolysis is complete. Six ml. of the mixture are then placed in a clean Shevky-Stafford centrifuge tube and centrifuged for 10 minutes at 2,000 r.p.m. The supernatant fluid is drawn off down to the 0.1 ml. mark on the tube, and the remaining fluid is mixed with a narrow glass rod. It is withdrawn with a capillary pipette and the entire 0.1 ml. spread on a clean glass slide. The slide is then scanned rapidly with the low power of the microscope. Microfilariae are easily visible even on

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cursory glance since they are unusually motile and since vision is not obscured by the presence of red blood cells as it is in an ordinary thick drop of blood. Sufficient white blood cells are present to accentuate the motion of the microfilariae. If a vaselined cover slip is used to prevent evaporation, motility is preserved for at least four hours and often as long as twenty-four hours at room temperature. Staining is usually unnecessary since the microfilariae are characteristic and since species differentiation can be made on epidemiological and clinical grounds. If desired, the smear may be thoroughly dried and stained with double strength Giemsa stain.

Recoveries by this method generally range from 90 to 105 per cent of the theoretical concentration. An experiment to determine whether a 1 to 1 dilution of the blood was sufficient to allow complete centrifugation of the microfilariae showed that essentially the same results were obtained whether the blood was diluted with ½, 1 or 1½ volumes of water.

The following results were obtained when the various methods of concentration were compared. Since it is frequently difficult to detect unstained immotile microfilariae and since we have found that many microfilaria will wash off the slide in the ordinary process of Giemsa staining, the final suspensions were mixed with Giemsa stain and allowed to dry before enumeration of the microfilariae.

METHOD	NUMBER OF MICROFILARIA FOUND IN 1/100 ML.	NUMBER OF MICROFILARIA EXPECTED FOR THEORETICAL YIELD
Thick smear	10	
Serum from clotted blood	4	
White cell layer of centrifuged blood	28	
Method of Fantham*	54	50
Method of Knott†	13	50
Method of Rivas‡	89	100
Author's method	152	150

* The Animal Parasites of Man. Fantham, H. B., Stephens, J. W. W., and Theobald, F. V. New York: William Wood and Company, 1920.

† *Knott, J.: Proc. Roy. Soc. Trop. Med. and Hyg.*, **33**: 191, 1939 (July).

‡ Human Parasitology. Rivas, D. Philadelphia and London: W. B. Saunders Company, 1920.

The concentration of microfilariae in our method is sufficiently quantitative to be used for the estimation of the number of microfilariae per ml. blood when they are present in very small numbers. From the point of time saved, the concentration is virtually much greater than the theoretical since larger quantities of the transparent hemolyzed fluid can be examined and since the motile microfilariae do not require the intensive searching necessary when immotile unstained or stained microfilariae are present. The method is convenient and adapted to the rapid routine examination of a large number of samples.

PORTUGUESE MAN-OF WAR STINGS: A CASE REPORT

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In tropical and semi-tropical waters, many swimmers have at one time or another come into rather disagreeable contact with jellyfish (Hydrozoa and Scyphozoa) and Portuguese men-of-war (Physalia). As a general rule, such contacts have led to painful local reactions but not to systemic reactions of any importance.

The following case demonstrates a rather unusual systemic reaction to the sting of a Portuguese man-of-war, of such severity as to actually endanger the patient's life.

The patient was a 23 year old sailor, who had previously been in good health. On 8 August 1943, while on liberty, he went swimming off of one of the beaches on the island of Oahu. About 0730 that day he was stung across the arms and thighs by what he and his companions identified as a Portuguese man-of-war. About 0900 he began to experience mild nausea and generalized muscular weakness. Shortly thereafter he began having spasms of the abdominal muscles, especially the recti abdominis.

He was admitted to an Army hospital at 0935, at which time his temperature was 98.6 (oral), pulse 96, respirations 24 and blood pressure 140/100. Examination revealed a well developed young white male who appeared very apprehensive and acutely ill. Across the anterior surface of the right arm and shoulder, and the right thigh were linear areas of redness which were not raised. The skin was not broken. Examination of the head and neck, lungs, heart, abdomen, lymphatic and endocrine systems was not abnormal. Intermittent spasms of the recti abdominis muscles were seen on inspection. Spasm of the diaphragm manifested itself by recurrent inspiratory gasping similar to that seen in hiccoughs. At this time, the patient was having only mild respiratory distress.

The clonic contractions began to increase in severity, becoming almost tonic in character, and spread to involve the muscles of the neck, shoulder girdle and both upper and lower extremities. The patient complained of rather severe pain in the muscles during the phase of contraction. Loud noises, bright lights and jarring of the patient's bed seemed to precipitate convulsive attacks, the reaction being similar to that seen in strychnine poisoning. The patient was entirely rational and showed no mental aberrations of any type.

By 1930 on 8 August, there was marked difficulty in breathing, respirations being slow and very labored, frequently interrupted by prolonged diaphragmatic spasms. Although there was no cyanosis, and the temperature, blood pressure and pulse rate were within normal limits, the patient appeared to be dangerously ill, with respiratory failure impending. Even at this time, he was alert and showed no other evidence of central nervous system depression.

The patient slept at intervals during that night, but even during sleep, the muscular spasms continued to be generalized and only slightly less intense than while the patient was awake. The persistence of these involuntary muscular contractions even during sleep was considered to be noteworthy.

On the following morning (9 August), the temperature, pulse, respiration and blood pressure were within normal limits. The clonic contractions were appreciably diminished, and the patient appeared to be in much better condition. However, in the afternoon of 9 August, the muscular contractions increased in severity, reaching the same intensity as noted on the previous day, again causing appreciable pain to the patient. There was no respiratory distress of significant degree at this time.

By the morning of 10 August, he had improved somewhat, but later in the day, increased severity of the contractions again noted. The spasms of the recti abdominis appeared to bother him most and he would flex his lower extremities and his trunk in order to obtain relief.

On the 11th, 12th, 13th, 14th and 15th of August, mild transient muscle twitching, involving particularly the recti abdominis, continued to be present.

By 16 August, the spasms and twitching had entirely ceased. The patient had no complaints. On 20 August 1943, he was discharged from the hospital completely free of signs and symptoms.

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TREATMENT

Upon admission to the Receiving Office, the patient was given 0.016 grams (one fourth grain) of morphine sulfate and 0.3 cc. epinephrine hypodermically by the Receiving Officer, who was of the opinion that the presenting symptoms were allergic in nature. Upon reaching the ward, the patient was given one gram of calcium chloride intravenously without effect on the muscular spasms. A similar dose of calcium levulinate was given intravenously later in the day, likewise without effect. Sodium phenobarbital by hypodermic was given at frequent intervals in doses of 0.065 and 0.13 grams (one and two grains), and this medication was continued until the spasms subsided. On the day of admission, it was necessary to give the patient 0.4 grams (six grains) of sodium amytal intravenously to diminish the severity of the muscular contractions. Medication with the barbiturates was felt to be of considerable value. The remainder of the treatment consisted of administration of hypertonic glucose solutions intravenously, adequate fluid intake by mouth and maintenance of nutrition.

DISCUSSION

Neurological examinations during hospitalization did not reveal any abnormal findings except for the involuntary muscular contractions. The eyegrounds showed no significant changes. A spinal puncture was not done at the outset because the danger of breaking off the needle in the spinal canal was felt to be a real one due to the convulsive movements of the patient.

Laboratory examinations done on the 8th and 9th of August gave the folowing information: (1) urinalysis: negative; (2) complete blood count: red blood cells 4,560,000; hemoglobin 14.9 grams, white blood cells 11,500, neutrophiles 75% of which 6 were stab forms, lymphocytes 21%, monocytes 2%, eosinophiles 2%; (3) blood Kahn: negative; (4) blood calcium: 14 mgm. %; (5) blood NPN: 37 mgm. %; (6) erythrocyte sedimentation rate: 3 mm. per hour.

The administration of calcium salts intravenously failed to relieve the muscle spasms, but the use of barbiturates appeared to be of considerable value in controlling them. The authors feel that the control of the spasmodic contractions was of great importance in preventing the patient from completely exhausting himself.

The question as to whether this was an hysterical reaction was considered. The staff psychiatrist concurred with the authors that the patient was a well-adjusted individual who had no abnormal psychoneurotic tendencies. This fact was further substantiated by subsequent interviews with the medical officer of the patient's organization.

It is interesting to note that Old (1) in reporting nine similar cases which occurred in the Philippine Islands, described the case of a fourteen year old boy who died "in hysteria" several hours after he had been released from medical observation on the day that the stinging occurred.

Stuart and Slagle (2) reported two similar cases occurring in Puerto Rico. They claimed favorable results from the use of calcium gluconate intravenously.

Wade (3) described the post-mortem findings in an individual who died a few minutes after having been stung on the lower extremity by a jellyfish. The findings were said to be those of generalized visceral congestion and status lymphaticus, the author further stating that the general impression gained was the same as that encountered in death from suffocation.

Several weeks after the patient's discharge from the hospital, information received from his unit medical officer indicated that he was getting along well without any recurrence of his symptoms and apparently without any residua.

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ANOPHELES PUNCTIMACULA D. & K. AS THE VECTOR OF MALARIA IN MEDELLÍN, COLOMBIA, SOUTH AMERICA[1,2]

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The present publication is concerned with the importance of *Anopheles punctimacula* D & K 1906 as the vector of malaria in Medellín and the neighboring locality of Itagüi in the Department of Antioquia of the Republic of Colombia. Medellín is located in the valley of the Rio Medellín at an elevation of 1540 meters above sea level, and has a mean temperature of 22°C. It is located 6° 15′ 6″ latitude north and 1° 28′ 59″ longitude west of the meridian of Bogotá.

METHODS

The spleens were palpated according to the technic of Boyd (1). Each thick film blood smear, after having been stained with Giemsa, was examined carefully for 10 minutes. The anopheline studies were carried out more or less according to the technics of Boyd (1) and Elmendorf (2). The P. D. I. spleens were classed as No. 1 of the Boyd Classification. The mosquito dissections were done according to the technic of Wilcox and Logan (3).

MEDICAL OBSERVATIONS

Malarial surveys of school children between the ages of 5 and 14 years and of persons of all age groups examined in their homes were carried out at different times of the year. These data are summarized in Table I.

The persons examined were approximately equally divided as to sex and essentially all were white. Owing to the low incidence of malaria in the area, great care was exercised in palpating the spleens considered as No. 1.

In the zone of Floresta among a total of 186 persons of all ages examined, a spleen index of 30 per cent and a parasite index of 17 per cent were encountered. The density of gametocytes in this sample was 9 per cent of the positive bloods. It was in this zone of the highest malarial incidence that the first infected stomach was encountered.

ENTOMOLOGICAL OBSERVATIONS

Four species of *Anopheles* have been found in the Medellín-Itagüi area during the 16 months of study. These are *Anopheles argyritarsis* Robineau-Desvoidy 1897, *Anopheles pseudopunctipennis* Theobald 1901, *Anopheles eiseni* Coquillet 1908, and *Anopheles punctimacula* Dyar and Knab 1906.

A. argyritarsis and *A. pseudopunctipennis* were identified by repeated observations on larvae, females, eggs and male terminalia, in accordance with the characters used by Komp (4) and Causey (5). The larvae of *A. pseudopunctipennis* always presented the post-spiracular tails, and the eggs were like those described by Rozeboom (6) from Panama. *A. eiseni* was identified by examinations of larvae, females, and male terminalia. Special interest and care was given to the identifications of *A. punctimacula* owing to the fact that the larvae breed in situations differed from those usually described for this species (see discussion). The identifications of this species were made by repeated examinations of larvae, pupae, male terminalia, females and eggs. The eggs of this species in Medellín are like those described by Kumm (7) from Costa Rica, all three of Kumm's types being represented.

For 16 months systematic captures of larvae and of adult female anophelines were made, the latter in human habitations as well as in stables and Magoon (8) traps. These data are summarized in Table II.

The stomachs of 177 female *A. punctimacula* taken exclusively from human habitations were examined for malarial cysts. One of the writers (Soto) found two stomachs which he suspected as having cysts, but unfortunately the preparations were destroyed. Later, Soto found in Floresta one

[1] The studies herein reported were conducted as a part of the program of the Departmento de Malariología of the Servicio Cooperativo Interamericano de Salud Publica, of the Ministerio de Trabajo, Higiene y Prevision Social of the Republic of Colombia.

[2] The authors wish to express their thanks and indebtedness to Col. W. H. W. Komp for checking the identification of *Anopheles punctimacula* and for his many helpful suggestions; and to Dr. M. F. Boyd for verifying the presence of malarial cysts on the stomach.

stomach with cysts which were verified by M. F. Boyd.

DISCUSSION

The data of the malarial survey of Medellín shows a low or hypoendemic incidence with a great predominance of *P. vivax*. The variations of the malaria during the year are not considered in this publication. The importance of the local anophelines as vectors of malaria is presented in the following paragraphs:

TABLE I

Parasite and spleen indices among 2186 school children and 1191 other persons of all ages in Medellín and Itagui, Colombia, during a period of 16 months

MALARIA INCIDENCE IN	PLASMODIUM PARASITES										
	Vivas		Falciparum		Mixed		Gametocytes		Total examined	Total positives	
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent		Number	Per cent
School children	167	95	22	12	13	7	8	5	2,186	176	8
All ages	124	92	11	8	0	0	43	32	1,191	135	11

	SPLEENS						
	I	II	III	IV	Total examined	Positives	
						Number	Per cent
School children	514	39	4	1	2,186	558	26
All ages	195	45	4	2	819	246	30

TABLE II

Anophelines captured in breeding places, houses, magoon traps and stables during a period of 16 months in the Medellín area

SPECIES	TAKEN FROM BREEDING PLACES		FEMALES CAPTURED IN MAGOON TRAPS AND STABLES		FEMALES CAPTURED IN HOMES	
	Number	Per cent	Number	Per cent	Number	Per cent
Argyritarsis	9,267	54	40	3	10	1
Punctimacula	4,909	29	1,093	96	816	98
Pseudopunctipennis	2,954	17	11	1	6	1
Eiseni	55	*	0	0	0	0
Totals	17,185	100	1,144	100	832	100

* Less than 0.5 per cent.

A. argyritarsis

This species was much the most abundant in the breeding places (54 per cent of all larvae collected). The types of breeding places were much the same as those described by Komp (4) and Rozeboom (9). The species was only very rarely found in human habitations (1 per cent of the female *Anopheles* taken in houses). These facts are in agreement with the observations of Earle (10) and Kumm, Komp and Ruiz (11). Simmons (12) and Simmons and Aitken (13) give summaries of the possible rôle of this species, as well as the others herein considered, as a malarial vector. We feel justified in concluding that in Medellín it is of no importance in the transmission of malaria.

A. pseudopunctipennis

This species constituted 17 per cent of the larval anophelines taken from the breeding places. It was commonly found together with *A. argyritarsis* in old abandoned borrow-pits, and also in pools of water containing algae. This species also was found in houses only very rarely (1 per cent of the female *Anopheles* taken in habitations). It also represented only 1 per cent of the total female anopheline catch in the stables and Magoon traps. *A. pseudopunctipennis* in Medillín would also seem to be of no importance as a vector of malaria.

A. eiseni

The occurrence of this species in the malarial zone of Medellín may be considered as purely coincidental. The larvae (55 in all) were found in a single breeding place in the San Antonio de Prado section, which has a very low spleen index. One

adult male was captured in a house in the more malárial section of Guayabal, indicating its probable presence as larvae near there.

A. punctimacula

The larvae of this species constituted 29 per cent of the total number collected in Medellín. Here, at a temperature of about 22°C., it commonly breeds in ground pools exposed to the sun, often very close to human habitations. Its breeding places are very numerous throughout the expanse of the nearly treeless, broad, flat valley of the Rio Medellín. It is also found in shaded, slow-moving branches, the banks of which are covered with herbaceous vegetation. According to Simmons (16), Covell (15) "states that the larvae are found in open pools, pools in connection with streams and in slowly running streams containing leaves."

In Panama, however, this species has been considered a "shade lover". Simmons (16) states: "In Panama *A. punctimacula* is found only in well shaded waters." Simmons also quotes a personal communication of November, 1935, from Dr. D. P. Curry, stating that "it is a jungle-breeding mosquito in Panama and the larvae are to be found in shaded ground pools." He further states, "Curry suggests that reports indicating that *A. punctimacula* in other countries breeds in open pools, rich in algae, may have resulted from confusion with the recently described *A. neomaculipalpus* Curry, (17), a very similar species, which breeds in such places." The writers have noted also that in certain lowland areas in Colombia this species is largely confined to well-shaded waters. As yet, at least, we do not have any evidence of its breeding in places fully exposed to the sun except in the highlands. Kumm, Komp and Ruiz (11) found it breeding in both sunny and shaded situations in Costa Rica, but no mention was made as to whether the collections from shaded situations were confined to the highlands. Since *A. punctimacula* seems to thrive at moderately high altitudes in the open sun as well as in shaded situations, there arises the question as to whether or not its limitation to well-shaded places in hot areas (if established generally) may not be due to a simple difference of temperature. This question may well warrant experimental study. The importance of this species as a local malarial vector is discussed in the following separate heading.

THE RÔLE OF A. PUNCTIMACULA IN MALARIA TRANSMISSION

A. Experimental infections

Darling (18) in Panama and Benarroch (19) in Venezuela obtained negative results. Simmons (20) obtained positive results, and Simmons (12) and Simmons and Aitken (13) summarize these results.

B. Natural infections

Benarroch (19) in Venezuela and Kumm, Komp and Ruiz (11) in Costa Rica obtained negative results. Simmons (16) found oocysts full of sporozoites in 1 of 6 females captured in Fort Sherman (Panama). Rozeboom (21) dissected 103 females from the Chagres River area and found one infected. Cadena (22) in Colombia examined 634 stomachs and 246 salivary glands. He called attention to the fact that 624 of the stomachs and 236 of the glands were dissected at a time when inexperience in the work made it impossible to judge accurately as to the results, and he stated that 3 of the stomachs may have had cysts, although he reported all results as negative. One of us (Soto) found well-developed cysts in one stomach among 177 examined, the presence of the cysts being verified by Dr. M. F. Boyd, and the result being herein reported for the first time.

C. Epidemiological evidence

Previous to 1910 *A. punctimacula* was suspected as a vector of malaria in Panama, according to Simmons (12). Darling (18) and Dyar (23) considered it of no importance as a vector. Larde y Arthes (24) considered it a vector in El Salvador because it was the only anopheline found in a zone which had a high rate of endemic malaria. Simmons (12) concludes that *A. punctimacula* "is an efficient vector of malaria at least in unsanitated regions in Panama." In the present study, we may state definitely that this species was the only one which frequented human habitations in Medellín during a 16 month period (98 per cent of the total number of female *Anopheles* taken in houses were of this species), in spite of the fact that one other species, *A. argyritarsis*, was nearly twice as abundant in the breeding places, and *A. pseudopunctipennis* was also very common. Also, 96 per cent of the female *Anopheles* taken in the stables and Magoon traps were *A. punctimacula*. Similar observations on the habits of the adults of this

species are recorded by Kumm, Komp and Ruiz (11) and by Rozeboom (21). The data presented in the previous pages seem fully adequate for concluding that *A. punctimacula* is the vector of malaria in the city of Medellín.

SUMMARY

1. As a result of a survey of 3,377 bloods and 3,005 spleens in the Medellín-Itagüi area of Antioquia, Colombia, 8 per cent of the bloods of school children, and 11 per cent of the bloods of persons examined from all age groups in their homes, had plasmodia, and 25 per cent and 28 per cent, respectively, of the spleens examined among the same groups were enlarged.

2. *Plasmodium vivax* was the only parasite which was encountered in significant numbers, although in a few bloods, *P. falciparum* was found.

3. *Anopheles argyritarsis*, *Anopheles pseudopunctipennis*, *Anopheles eiseni*, and *Anopheles punctimacula* were the only anophelines found in the area during 16 months of study.

4. *Anopheles punctimacula* was the only species which frequented human habitation, and also the only one found to be naturally infected.

5. It is of importance in the planning of control work that this species was found in breeding places far removed from wooded land, in urban water holes fully exposed to the sun.

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TWO EXOGENOUS CASES OF VISCERAL LEISHMANIASIS (KALA-AZAR) IN THE UNITED STATES WITH NOTES ON CULTIVATION OF LEISHMANIA DONOVANI IN VITRO[1]

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Cutaneous and visceral leishmaniases are widely distributed in certain parts of the world; however, up to the present time, no endogenous cases and only a few exogenous cases of either type of leishmaniasis have been reported from the United States [Craig and Faust (2), Dwork (3), Mathieson and Watson (6), Packchanian (8), and Price and Myer (9), Strong (11), Wenyon (12)].

The first exogenous case of visceral leishmaniasis in North America was that of a Chinese student, aged 29, who entered the United States from China, September 3, 1935, as a student at the University of Minnesota. On December 15, 1935 (107 days after his entrance into the United States) he became ill and was admitted to the hospital with a final, proved diagnosis of kala-azar [Mathieson and Watson (6)].

The second case, hitherto unpublished, personal communication with one of the writers (A. P.), was that of an Italian boy who had apparently contracted the disease in Italy and manifested the clinical symptoms in Boston, Massachusetts. The case was diagnosed as kala-azar, and *Leishmania donovani* was cultured from the patient [McKhann (5)].

The third case was that of a 15-year-old Filipino girl, a former resident of Calcutta, India. One year after entering the United States, she was admitted to the Presbyterian Hospital in New York City, July 20, 1943. The diagnosis of kala-azar was based chiefly on the demonstration of Leishman-Donovan bodies in smears from the sternal bone marrow [Rose (10)].

A fourth patient, a 23-year-old Indian seaman, was admitted to the U. S. Marine Hospital in New York City in August, 1943. His illness was finally diagnosed as visceral leishmaniasis [Price and Myer (9)].

The fifth and sixth cases of exogenous visceral leishmaniasis were diagnosed recently by the writers and are described in this report. These patients were native Indian seamen from Assam, India, who arrived separately in San Francisco, early in 1944, aboard Dutch steamers.

CLINICAL AND LABORATORY FINDINGS

Our first patient, a 40-year-old Indian seaman, was admitted to the U. S. Marine Hospital on his arrival at San Francisco, California, on March 11, 1944, with a tentative diagnosis of malaria. He complained of fever, chills, headache, and diffuse abdominal discomfort, all of approximately four weeks' duration. He had shipped from Calcutta, presumably in good health, but while at sea had noticed fever and malaise. Physical examination revealed moderate emaciation, slightly enlarged liver, and markedly enlarged spleen, which extended approximately eight centimeters below the costal margin (fig. 1A). The temperature was high and irregular, usually with one but at times with a so-called "double rise", or two peaks daily (fig. 2).

The erythrocyte count was 2,500,000, and the leukocyte count varied from 2,700 to 3,400 cells per cubic millimeter. Blood smears for malarial parasites and the agglutination tests for typhoid, typhus, and undulant fever were negative.

After two weeks in the hospital, the temperature continued to rise daily and at times reached 104°F. (40°C.). A sternal puncture was made during this period. A second sternal puncture was performed on April 21, 1944. Smears made from both specimens were stained by a combination of the Wright-Giemsa stains and showed many aflagellar forms of Leishman-Donovan bodies, both intracellularly and extracellularly. Each aflagellar form of the parasite showed two distinct structures, i.e., a relatively large, ovoid, deeply stained, basophilic,

[1] The writers wish to express their thanks and gratitude to Dr. E. L. Gardner and Dr. Joseph Moore of the U. S. Public Health Service, and to Capt. M. A. Krupp of the U. S. Army for their kind cooperation and aid in this study, and to Mr. Robert Sage of the University of Texas, for his skillful photomicrography.

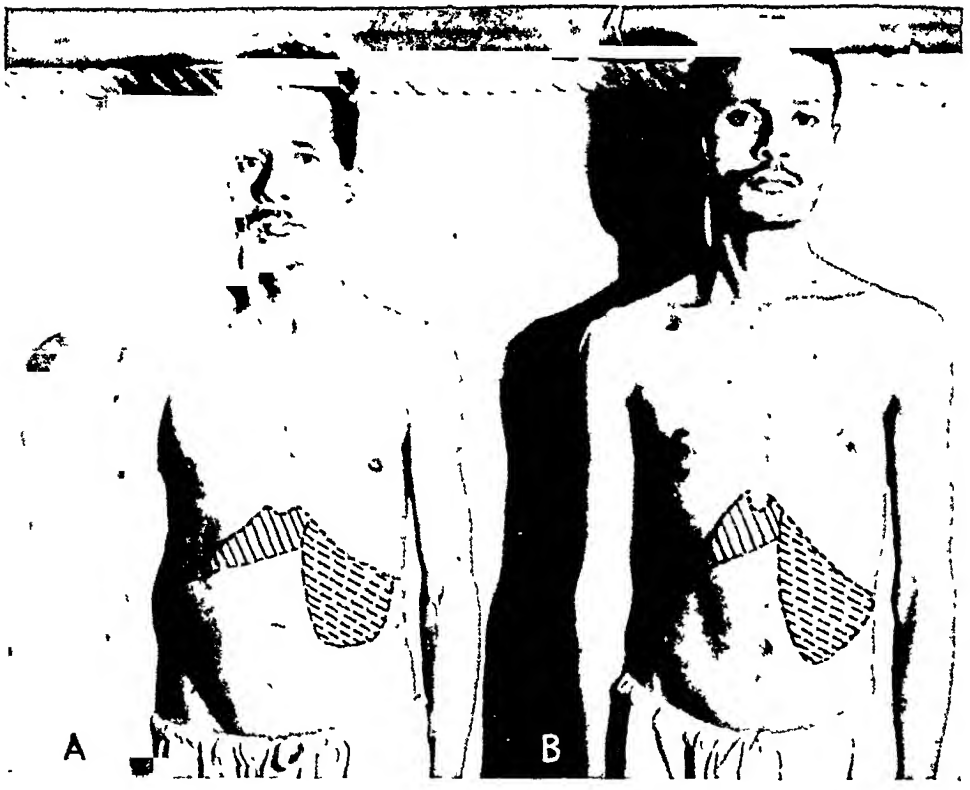

FIG 1 KALA-AZAR IN TWO ADULT INDIANS, DIAGNOSED IN THE UNITED STATES (EXOGENOUS CASES)
The patients were photographed toward the termination of the chemotherapy. The pencil tracing indicates the degree of enlargement of the liver and of the spleen

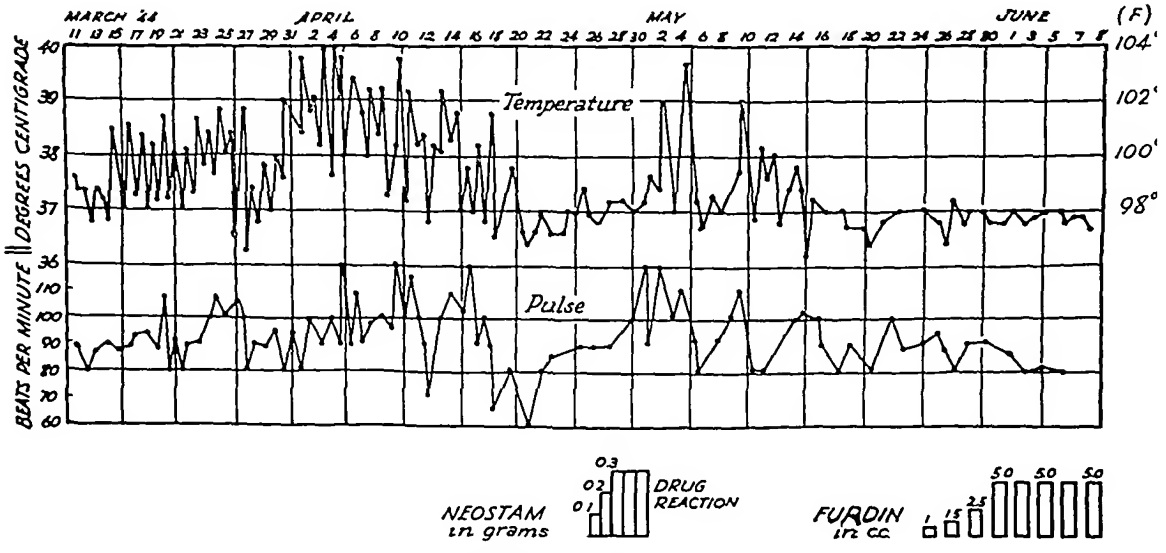

FIG. 2. TEMPERATURE CHART OF KALA-AZAR

peripherally located nucleus and a rod-shaped, more deeply stained kinetoplast (fig. 3).

In addition, on April 21, 1944, the following so-called "presumptive tests" for kala-azar were performed: (a) Brahmachari's serum-globulin or water test, (b) Napier's aldehyde or formol-gel test; and (c) Chopra's antimony test [Craig (1)]. All of these tests gave positive reactions.

On April 21, 1944, a specimen of bone marrow, obtained by the second sternal puncture, was inocu-

lated into several blood agar tubes containing 30 to 50 per cent of defibrinated rabbit's blood (N. N. medium with less than the customary amount of blood). The tubes were rubber capped and incubated at room temperature [Packchanian (7)]. Five days later (April 26, 1944), several tubes of the culture were found to be positive for motile, leptomonad flagellates which had the morphological and cultural characteristics of *Leishmania donovani*. Eight days after inoculation (May 1, 1944), the remaining tubes of cultures were found positive for flagellates. Colonies were distinctly visible on the slanted portion of some of the blood agar tubes, and the water of condensation was swarming with free swimming flagellates and rosettes. The second generation revealed almost identical cultural forms (fig. 4) which were found likewise in subsequent generations. The strain was maintained *in vitro* and subcultured until September, 1944.

On the same day that the second sternal puncture was made, about 10 cc. of blood were removed from the patient's arm; the blood was defibrinated and from 0.5 to 1.0 cc. was introduced into several blood agar slants (N. N. medium). These tubes were likewise rubber capped and incubated at room temperature (23° to 25°C.) and examined at various intervals. Positive growth of flagellates was noted in one tube after 16 days, in others after 19, 26, and 36 days respectively, while the remaining four tubes showed no growth. The growth of flagellates in some of the tubes was less heavy than that obtained from bone marrow at the same time and on identical culture medium; however, the subcultures yielded a rich growth.

The flagellates which had been cultured, both from the bone marrow and from the circulating blood, stained well with a combination of the Wright-Giemsa stains. They revealed all morphological characteristics of *Leishmania donovani*. A long flagellum could be traced from the kinetoplast, which was situated at the blunt anterior end of the parasite. There was no evidence of an undulating membrane. The nucleus was rather large and was located either centrally or toward the posterior end (fig. 4).

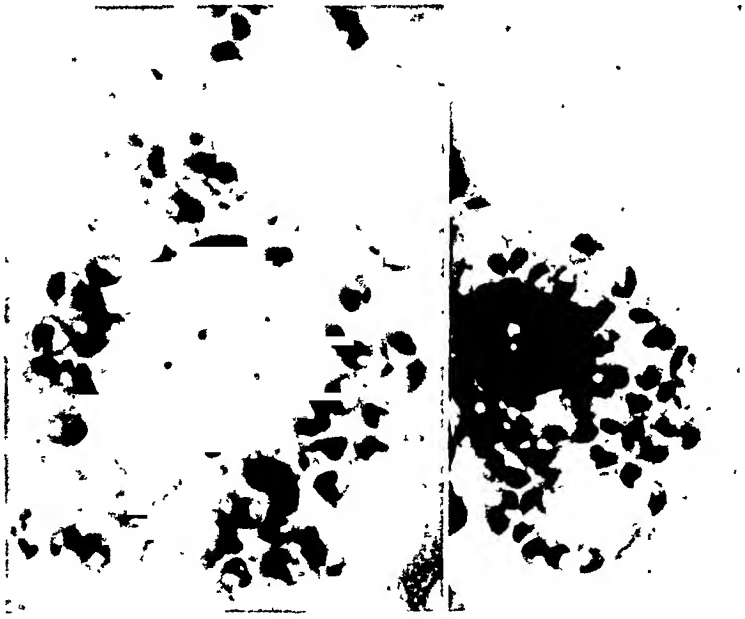

FIG. 3. LEISHMAN-DONOVAN BODIES OR AFLAGELLAR FORMS OF LEISHMANIA DONOVANI FROM BONE MARROW OF PATIENT NO. 1 OF KALA-AZAR (× 1600)

Our second patient was a 25-year-old Indian seaman, who had lived also in Assam, India, and had shipped from Calcutta. He entered the U. S. Marine Hospital at San Francisco, California, on April 10, 1944, complaining of chills and fever which had been occurring daily for a month prior to his entry, and of a vague pain in the left upper quadrant of the abdomen. Physical examination revealed that the liver was moderately enlarged and that the lower border of the spleen extended down to the level of the umbilicus (fig. 1B). The blood count showed 3,500,000 red blood cells and 3,500 white blood cells respectively per cubic millimeter. Tests of the urine were negative. Smears

for malaria were repeatedly negative, and agglutination tests for typhois, paratyphoid, and undulant fever likewise were negative. Because of the similarity of his symptoms and his clinical picture to our first patient, and because his fever also continued to run irregularly, the presumptive tests for kala-azar were performed. These all gave positive results. Leishman-Donovan bodies were demonstrated in smears from the sternal bone marrow.

FIG. 4. CULTURAL OR FLAGELLAR FORMS OF LEISHMANIA DONOVANI, COMBINATION OF WRIGHT-GIEMSA STAINS (× 1600)

TREATMENT

Both patients were treated with stibamine glucoside (neostam).[2] The drug was administered intravenously in gradually increasing doses, starting with 0.1 gram and an additional 0.1 gram was given with each successive dose until 0.3 grams were administered daily. The reaction of the first patient, however, became so severe after five injections that the drug was discontinued. Fuadin (sodium antimony biscatechol disulfonate of sodium) then was tried, and although this drug is supposedly less efficacious than the pentavalent antimony compounds, he improved rapidly and steadily and without further reaction. When he was returned to India from the hospital, he was clinically well but the presumptive tests still were positive.

The second patient received a complete course of treatment in 11 days with a total of 2.8 grams of neostam. Clinically, his improvement was marked, but because his presumptive tests remained positive, he was given a second course of neostam. At the time of his departure, he showed no clinical symptoms; the spleen was no longer palpable and the presumptive tests for kala-azar were negative.

DISCUSSION

For an absolute diagnosis of visceral leishmaniasis, it is essential to demonstrate: (a) the aflagellar forms, Leishman-Donovan bodies, in smears made from the bone marrow or blood; and (b) the flagellated forms grown *in vitro* from bone marrow or blood on suitable medium.

Both *Trypanosoma cruzi* and *Leishmania donovani* produce aflagellar forms or so-called Leishman-Donovan bodies that are morphologically almost identical in the vertebrate host. On the other hand, *L. donovani* can be readily distinguished from *T. cruzi* in cultures because the former occurs in the leptomonad form which lacks an undulating membrane and the latter appears as a

[2] Neostam (stibamine glucoside) can be purchased from Burroughs and Wellcome, New York City Branch, and neostibosan (ethylstibamine) from Winthrop Company, New York City.

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REPORT ON A CASE OF BALANTIDIASIS[1]

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Balantidiasis is generally considered to be a rare infection in man. Recently, Young (1) reviewed the literature and estimated a total of only 41 cases reported in continental United States. The states represented in the review included Arkansas, Iowa, Louisiana, Maryland, Massachusetts, Minnesota, Mississippi, North Carolina, New Mexico and Washington. In addition, Craig and Faust (2) claimed to have observed thirty six cases in the course of thousands of stool examinations.

In view of the comparative paucity of human balantidiasis in this country, it appears to us the present case is worth reporting. Thus far, no authentic cases have been reported from the state of Missouri.

REPORT OF THE CASE

B. L., white female, 35 years of age was admitted to Barnes Hospital with complaints of cramping, epigastric pain, lower abdominal distress, and diarrhea almost daily for the past four months. There had been little associated nausea and no vomiting. She had lost ten pounds during the same period. The pain was described as being colicky in nature, usually occurring one to two hours after meals, but had occurred at any time during the 24 hours, frequently keeping her awake at night. There were no particular food intolerances; all foods seemed to cause some distress. Stools were soft, frequently watery containing little mucus; no fresh blood was ever noted.

Past history: There was nothing to indicate that the patient's previous history bore a particular connection with the present condition. On the contrary, she had previously suffered from constipation. She has, however, always been thin and underweight, and has had numerous illnesses for which she was admitted to the hospital 6 times previously.

Social history: The patient lives in a small southwestern town in Missouri. Her occupation is pharmacist and she owns and operates a drug store. She stated emphatically no possible close contact with hogs and had never partaken any unprepared porcine meat. She had eaten daily in a restaurant run by her mother.

Physical examination: A poorly nourished and delicate woman. Skin was clear and no general glandular enlargement was present. Blood pressure was 104/70. The abdomen was scaphoid and the right kidney was palpable but not tender. There was a considerable diffuse lower abdominal tenderness without guard. Lungs and heart were negative.

Proctoscopic examination: It was entirely normal except some pallor and an increased amount of mucus.

Laboratory examination: Urine was clear and normal. Blood count: there were 4,080,000 red blood cells per cu. mm.; 4600 white blood cells; 76 per cent of hemoglobin and normal Schilling. Blood chemistry: non-protein nitrogen was 11 mgm. per cent and sugar 54 mgm. per cent. Kahn test was negative. Stool examination revealed the presence of trophozoites of *Balantidium coli* and no cysts were found. Stools were negative for typhoid and dysentery groups. Roentgen ray examination of chest was negative and gastrointestinal series revealed nothing but somewhat hypotonic and redundant colon.

Treatment: The patient was treated with carbarsone 0.25 grams twice daily for ten days. Subsequent stool examinations made for a period of two months were repeatedly negative for *Balantidium coli*.

COMMENT ON THE CASE

Though the pathogenicity of *Balantidium coli* was generally accepted by clinicians, symptomatology greatly varies. Many individuals exhibit no apparent symptoms such as in some cases of amebic carriers, but in a vast majority of instances protracted diarrhea is known to occur as exemplified by the present case. In severe infection, it closely resembles amebic dysentery and dysentery with accompanying ulcerative processes may supervene. The fact that no other causes could be detected and that the diarrhea promptly disappeared following the treatment strongly indicated that *Balantidium coli* was responsible for the condition of this patient.

It is generally believed that man acquires the

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infection through direct or indirect contact with hogs rather than from human sources. Craig and Faust claimed that in 25 per cent of reported cases of the infection gave a history of direct contact with the animals leading to hand to mouth transfer of cysts. Trophozoites of the organisms are seldom considered the infective stage probably because of their brief extracorporal viability. However, Rees (3) has found that trophozoites were often able to survive for ten days at room temperature under anaerobic condition. According to Hegner (4) they are capable of passing unharmed through the stomach and reaching the caecum of guinea-pigs. In view of the apparent absence of contact with hogs, as stated by the patient, we may assume that a probable source of the infection may be traceable to the presence of human carrier. However, the low incidence of human infection tends to preclude the possibility of man being an important source of transmission, though carriers among food handlers may be responsible for a small percentage of cases. An attempt to secure stool sample of employees at her mother's restaurant was not materialized; hence this possibility was not taken into consideration for time being.

Houseflies as vectors of the infection may be considered important, though no experimental studies have ever been made as to the exact rôle of houseflies in the transmission of balantidiasis. It is known that houseflies are capable of flying several miles a day. A record flight of 13 miles within a few days has been reported. Craig (5) believes that cysts of *Endamoeba histolytica* adhering to the bodies of flies are usually destroyed by desiccation but those remaining on the feet may contaminate food while still in a viable condition by walking over moist food stuff. Root (6) found that cysts of some human protozoa remained viable for a considerable time in the guts of flies, the majority dying in about 15 hours, but some remaining alive as long as 49 hours. We may postulate, therefore, that since the patient lives in a small town not far from surrounding rural areas, it is quite possible that migrating flies might have served as "passive carriers" thus contaminating food and drink. This may occur in localities where sanitary conditions are such as to allow flies access to fecal matter of infected hogs. There may also exist some other as yet unknown domestic reservoirs of the balantidia.

Rats and mice as probable reservoir hosts have been eliminated on the basis of the fact that balantidia have been rarely found in their intestines in nature. Attempts to infect laboratory bred rats and mice by various methods of inoculation with culture containing numerous trophozoites of *Balantidium coli* isolated from the feces of the patient resulted negatively in all instances. A detailed study on the experiment will be reported later. Since no cysts were found in the culture medium, we may say that the transmission was not possible by the use of trophozoites at least in our study.

The efficacy of carbarsone in the treatment of balantidiasis appears to be fairly well established. Young and Burrow (7) obtained very favorable results in all the cases treated with the drug. Recently, however, DeLanney and Beahm (8) reported that the treatment with carbarsone followed by oil of chenopodium gave only temporary relief while diodoquin in dosage of ten 0.25 grams a day for 20 days resulted in the absence of relapse after 18 months. Since no specific chemotherapeutic agent is known at present, it seems necessary to follow up a number of cases with periodical examination over a long period of time in order to determine the extent of specificity of the drug employed. This has been accomplished by Young and Burrow in their seven cases.

SUMMARY

1. The first authentic case of balantidiasis in man is reported from the state of Missouri and probable sources of infection discussed.

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FRESH LABORATORY MATERIAL FOR TEACHING MEDICAL PARASITOLOGY

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With the increased interest in parasitology there has developed the problem of obtaining an adequate supply of laboratory material for teaching purposes. Unfortunately, in the effort to obtain prepared slides and other fixed material, too frequently we have lost sight of the fact that *more valuable fresh living* material may be available locally. Fixed and stained material is essential to illustrate histopathology, to set forth the distinguishing characters of certain parasites (notably malaria), to illustrate forms which cannot be obtained in the living state, and to supplement the study of living material. It seems unfortunate, however, to use only fixed and stained material to illustrate diagnostic stages which the physician will rarely, if ever, see in such condition thereafter. However valuable a hematoxylin stained smear of *Endamoeba histolytica* may be to illustrate the morphology of the trophozoite, it is much less important than the fresh living trophozoites of any parasitic amoeba in the training of medical students. Actually the student, in his clinical contacts thereafter, will be called upon to recognize the forms in feces, probably without the facilities or opportunity of making hematoxylin preparation. If time permits, he may make his own hematoxylin stained preparation from the fresh material in class as he or his technician might do later in the clinic. What has been emphasized here with reference to *E. histolytica* holds with varying degrees for other forms studied.

Accordingly, an attempt is made here to outline some of the opportunities for obtaining fresh material to illustrate some of the commoner parasites of man. The suggestions offered grow out of the courses developed in the School of Hygiene and Public Health of the Johns Hopkins University under the direction of Dr. W. W. Cort and the late Dr. Robert Hegner. Our concern is first to obtain fresh living organisms for study. Usually species from man are to be preferred but where parasites from man are not available recourse is had to closely related forms from lower animals. More individual effort is required on the part of the teaching staff to provide fresh living material than in issuing permanent slides but the results are easily worth that extra effort.

I. INTESTINAL PROTOZOA

With the intestinal protozoa more than with any other group fresh living material is important in teaching the student the problems of microscopical diagnosis. One or more of the following sources may be utilized.

A. Service Laboratories or Clinics (Hospitals, Health Department Laboratories, Private Clinical Laboratories). Any or all of these services may be able to provide fresh material for class study. The dependability of such sources will rest on a number of factors such as 1) The prevalence of infection in the clientele served, 2) The number of routine examinations, and 3) the degree of personnel cooperation and proximity of the laboratory. In our experience such services have been extremely helpful to us but because of the low prevalence of infection in this area we have not been able to depend upon receiving a specified organism on any given day.

B. Staff, attendants, etc. The examination for intestinal protozoa of staff, assistants, graduate students, animal attendants and others associated with the department has never failed to provide one or more dependable sources of one or several of the intestinal protozoa.

C. Students. In almost any class one or more of the students is likely to harbor intestinal protozoa. If sufficient help is available a routine examination will reveal those whose feces are of teaching value. However, the students may be assigned the task of examining their own feces under supervision after they have had some preliminary study.

D. Animals. Representatives of the genera of intestinal protozoa which occur in man may be found in a variety of animals and in some cases the species infecting man may be found. The following list is given to illustrate the type of material which may be available; only forms similar to the human species which are readily found are listed;

forms very different from human species are noted if they are likely to be conspicuous. For more complete listing see Hegner, Root, Augustine and Huff (pp. 38–41).

1. Primates (monkey and chimpanzee) (feces):
   Balantidium coli
   Endamoeba histolytica
   E. coli and other species
   Iodamoeba sp.
   Trichomonas hominis
   Giardia lamblia (not very common)
   Note: *Troglodytella abrassarti* (ciliate) may be conspicuous
2. Pig (caecum). Material may be obtained at almost any abattoir killing swine:
   Balantidium coli
   Amoebae (not readily found)
   Trichomonas suis (not readily found)
3. Laboratory Rats (caecum, less easily seen in feces):
   Giardia muris (rats on dog chow may be less heavily infected than when on a grain diet).
   Trichomonas muris
   Chilomastix bettencourti (not common)
   Retortamonas sp.
   Endamoeba muris
   Note: *Hexamita muris* is conspicuously present.
4. Laboratory Mouse (caecum or intestine, less easily seen in feces):
   Giardia muris
   Trichomonas muris
   Amoebae
   Note: *Hexamita muris* is conspicuously present.
5. Guinea pig (caecum)
   Giardia caviae
   Trichomonas caviae
   Endamoeba cobayae (not as conspicuous as the flagellates)
6. Rabbit (feces)
   Eimeria stiedae (coccidia)
7. Chickens, frogs, snakes, cockroaches, and various other non-mammals may also be used as effective sources of intestinal protozoa.

E. Cultures. In general, intestinal protozoa from cultures are much less satisfactory for teaching purposes than the same species from the natural environment. However, *Endamoeba histolytica* trophozoites from cultures effectively illustrate the typical movement and are particularly useful if no other source of living amoeba is available. The large mass of starch granules in these amoebae, however, mask much of the characteristic morphology of the organism. It is not always easy to start the culture from feces but an experienced technician can quite easily maintain, by subculturing, a strain once it is established.

The pathogenic amoeba of snakes, *E. invadens* (see Geiman and Ratcliffe, 1938 and Larsh, 1944), is morphologically indistinguishable from *E. histolytica*. Since it readily grows in culture and is actively motile at room temperature, it is an effective substitute for *E. histolytica* in teaching the identification of the latter form.

II. BLOOD PARASITES

There is no adequate substitute for stained blood mears in the routine laboratory diagnosis of malaria, but if preparations of human malaria are not available those of bird malaria may be used effectively. Even when stained smears of human malaria are available it is of value to demonstrate some stages of the living parasites; this may often be accomplished more easily with bird malaria. Species of bird malaria, such as *Plasmodium cathemerium*, are particularly useful for demonstrating exflagellation. This may be seen within 20 to 40 minutes in a vaseline sealed fresh drop of blood under oil immersion or even under 4 mm. objective. Malaria infections are common in a wide variety of birds but the English sparrow is perhaps, in general, the most convenient source of bird malaria in nature and such infections may be transmitted by blood inoculation to both sparrows and canaries.

Trypanosoma rotatorium and microfilaria of *Folcyella* sp. are both common in frogs from Florida and Louisiana. Microfilaria may also be found in both dogs and crows in the south and have been reported to be common in the snowshoe rabbit in Minnesota. *T. lewisi* occurs in wild rats and may be maintained in laboratory rats by intraperitoneal subinoculation.

INTESTINAL HELMINTHS

As in the case of the intestinal protozoa the natural infections in man may be utilized when available. However, except in the South, helminths are less commonly encountered than are the protozoa so that we have come to depend more heavily on animal infections for the laboratory demonstrations of the intestinal helminths.

A. Tapeworms (Cestodes)

Laboratory mice are good sources of *Hymenolepis nana* infection; many but not all colonies will be found to be naturally infected. Eggs may be obtained from feces or, at necropsy, from the terminal

proglottids of the worms. Once the parasite is established in the colony infection may be maintained relatively easily by adding young mice to a stock colony at two to four week intervals. A convenient procedure is to heavily mark with dilute eosin the young mice added to the cage and at that time remove and discard those from which the color has completely disappeared. The litter in such cages should not be changed any oftener than once a week at the most.

The eggs of the cat tapeworm, *Taenia taeniaformis* and the dog tapeworms, *Taenia pisciformis*, *Multiceps multiceps*, and *M. serialis*, are indistinguishable from those of *Taenia saginata* and *T. solium*. Eggs may be recovered from the feces or, at necropsy, from the terminal proglottids of the worms. The cat tapeworm will more likely be found in cities where rats are common; the above dog tapeworms are more likely to occur in rural dogs or those from small towns, but the double-poured tapeworm, *Dipylidium caninum*, occurs commonly in the city dogs.

Cysticeri may be demonstrated in the livers of wild rats or even some colonies of laboratory rats (*Cysticercus fasciolaris* = *Taenia taeniaformis*), the mesenteries of cotton-tail or jackrabbits (*Cysticersus pisciformis* = *T. pisciformis*), or the subcutaneous tissues of the same rabbits (*Coenurus serialis* = *M. serialis*).

B. Round worms (Nematodes)

1. General structure of adult worms. The general structure of adult nematodes may be illustrated with nematodes of insects; the oxyurids from the rectum of the American roach, *Periplanta americanus*, are particularly good for this purpose.

2. *Trichinella spiralis*. This is, perhaps, the easiest nematode infection to maintain in the laboratory. Every mammal is susceptible to infection and the skeletal muscle of any infected mammal will serve as a source of infection. Infective muscle may be maintained in good shape in a refrigerator for weeks. Dump rats or garbage-fed hogs are good sources of infection, but it is easiest to obtain the original infection from one who has it in laboratory animals. Rats will, after 24 to 36 hours starvation, readily eat infected meat. The typical infective muscle stages can be demonstrated at necropsy in the diaphragm or other skeletal muscle any time after the third week of the infection throughout the life of the rat.

3. Hookworms. *Ancylostoma caninum* is quite common in half-grown and even mature dogs, particularly stray dogs, in many parts of the United States. They are common in young puppies in crowded kennels and *Uncinaria stenocephala* is common in crowded fox farms. *Ancylostoma braziliense* may be found in both cats and dogs in southeastern United States. The eggs of these forms are only slightly larger than those of *Necator americanus* and otherwise similar to them in feces. Cultures of feces with equal parts of sterile sand or charcoal at room temperature (kept moist) will produce infective larvae in 7 to 12 days. Such larvae may be isolated by suspending the culture in a stoppered funnel of warm water (Baermann apparatus), the larvae being drawn off through the stem in an hour. Several hundred to several thousand *A. caninum* larvae placed on the skin or injected subcutaneously in a puppy will produce a good infection in three weeks.

4. *Strongyloides*. Every colony of rhesus monkeys or chimpanzees we have examined has had one or more *Strongyloides*-infected animals. Eggs or first stage larvae are found in fresh feces; infective larvae may be found in 36 to 48 hour cultures (prepared as hookworm cultures) but since indirect development usually occurs free-living adults will be found in 2 to 7 days and infective larvae in 3 to 6 days.

Wild rats in many areas and some colonies of laboratory rats will be found to harbor *Strongyloides ratti*. Except for the preponderance of eggs, rather than first stage larvae, which occur in the feces they are not unlike human or other primate *Strongyloides* for class use; development is usually direct with infective larvae in 36 to 48 hours.

5. *Ascaris lumbricoides*. The ascaris of pigs is morphologically indistinguishable from human ascaris. They can usually be obtained from the Federal Meat Inspector or butcher wherever swine are slaughtered. Normal eggs may be obtained from the proximal portion of the uterus of the female worms. Developmental stages can be obtained by maintaining them in water with 1% formalin or potassium bichromate at room temperature; larvae will develop in them in 10 to 15 days.

C. Trematodes (Flukes)

There are no commonly available trematode infections in the United States which are similar to the species occurring in man. However, living trematodes may be demonstrated in a number of animals. The frog is a particularly useful animal

for this purpose since trematodes may be found in the rectum (*Diplodiscus temperatus*), intestine (*Cephalogonimus* sp. most common) the urinary bladder (*Gorgodera* sp. and *Gorgoderina* sp.), in the lungs (*Pneumonoeces* sp. and related forms), and in the subcutaneous tissue and mesenteries (metacerceria of *Clonostomum attenuatum*). The worms are most easily demonstrated by dissecting the frog. Some species, particularly the lung flukes, will discharge their eggs when stored in tapwater about an hour; if eggs are not discharged they may be obtained by teasing the worm apart; miracidia will hatch in a few minutes from the eggs of the rectal and bladder flukes.

Fresh water snails harbor a variety of larval trematodes. The cercaria may emerge in large numbers when the snails are placed in tap-water in milk bottles or even small containers. If 1 to 5 snails are put in each container, it is easier to detect those which are infected. The cercaria should be visible to the naked eye or with a hand lens in 12 to 24 hours and may be transferred by means of a pipette to slides for microscopical study.

Redia or daughter sporocysts containing cercaria may be dissected out of the digestive glands of these same snails.

IV. SUMMARY AND LITERATURE

It is hoped that one or more of the above suggestions may prove helpful to some of those who are having difficulty in obtaining laboratory material for teaching purposes. No attempt has been made to offer a complete list of possible forms available or to outline laboratory procedures. Only two specific references (concerning *E. invadens*) and a few more general references are appended.

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LEPROMIN SKIN TESTS IN BOECK'S SARCOID

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In 1943 Pardo-Castello and Tiant, of Cuba, presented an excellent review of the immunologic types of leprosy and the varying responses to lepromin skin tests (1). During the discussion Pardo-Castello stated that almost all apparently healthy adults in Havana give a positive lepromin test, and suggested that the cases of Boeck's sarcoid found in Cuba might be instances of atypical leprosy. These statements suggested to us the intriguing possibility that the Boeck's sarcoid, or sarcoidosis, found in the United States might be attenuated or modified leprosy.

Through the courtesy of Dr. Pardo-Castello, we obtained 2 cc. of lepromin for testing a series of proven cases of Boeck's sarcoid. Dr. George Harrell developed the same idea of testing sarcoid cases with lepromin after conferring with Pardo-Castello, but obtained his lepromin from the leprosarium at Carrville, La.

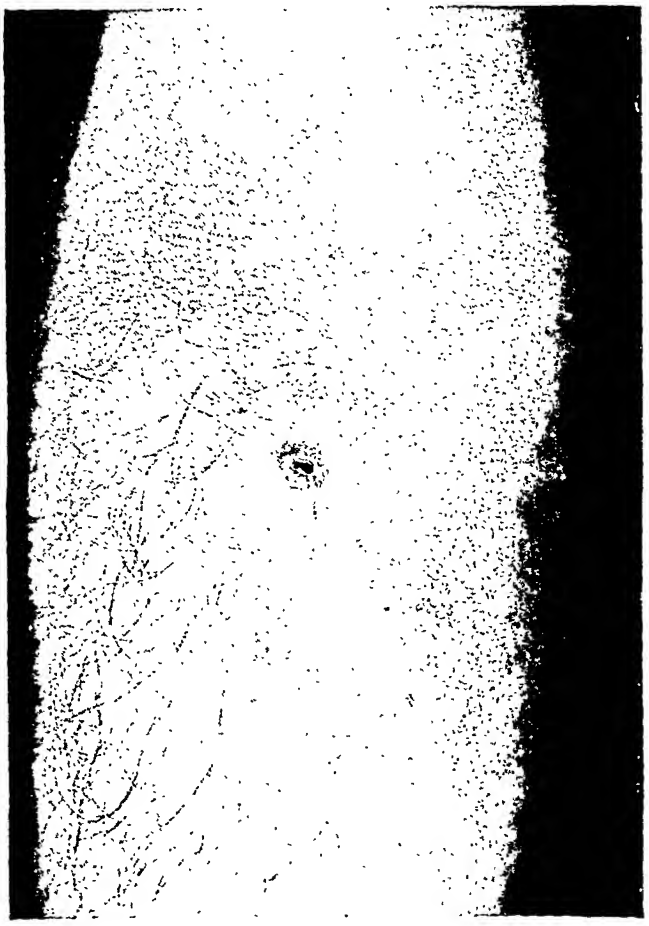

FIG. 1. A POSITIVE LEPROMIN TEST ON THE FOREARM OF A PATIENT WITH ACTIVE PULMONARY TUBERCULOSIS
The photograph was made fifteen days after the lepromin test

The lepromin furnished us by Dr. Pardo-Castello was an opalescent fluid which contained a suspension of very small fragments of tissue and whole or partly broken but still acid fast bacilli of *Myco-*

bacterium leprae. The material had been sterilized by heat.

The skin test was performed by injecting 0.1 cc. of the lepromin intradermally in the skin of the ventral surface of the right forearm. The test was controlled by injecting into the corresponding area on the left arm 0.1 cc. of a 1:1000 dilution of old tuberculin.

There was practically no immediate reaction to the lepromin, but usually after twenty-four to forty-eight hours an erythematous halo, with or without infiltration, appeared and persisted from two to seven days. The true lepromin reaction begins between the seventh and fourteenth day as a small nodule which gradually reaches a maximum size between the third and fourth weeks. Often an area of central necrosis develops in the nodule which requires several weeks to heal (fig. 1).

TABLE 1

CASE	DISEASE	OLD TUBERCULIN	LEPROMIN
1	Sarcoid	Negative	Negative at 25 days
2	Sarcoid	Negative	Negative at 30 days
3	Sarcoid	Negative	Negative at 30 days
4	Sarcoid	Negative	Negative at 30 days
5	Sarcoid	Negative	Negative at 30 days
6	Sarcoid	Negative	Negative at 14 days
7	Sarcoid		Negative at 30 days
8	Sarcoid	Negative	Positive at 21 days
9	Sarcoid	Positive	Positive at 14 days
10	Sarcoid	Positive	Positive at 30 days
11	Pulmonary tuberculosis	Sputum positive	Positive at 15 days
12	Pulmonary tuberculosis	Sputum positive	Positive at 21 days
13	Normal	Negative	Negative at 14 days

Ten cases of Boeck's sarcoid were tested with lepromin and nine controlled by tuberculin. Several of these patients were included in the original series of cases reported by Harrell (2) from this clinic. In all cases the clinical symptoms were typical, and the diagnosis had been confirmed in each instance by a biopsy.

The lepromin was negative in seven of the cases of sarcoid (table 1), and the tuberculin was negative in six of the seven. The lepromin was positive in three cases; the tuberculin was positive in two of these cases. Two cases of active tuberculosis gave a definite positive lepromin test.

It is of interest that Case 9 gave a negative tuberculin test in 1934 when his disease was active. In 1944 all evidence of sarcoid had disappeared; the tuberculin was then positive and so was the lepromin. In other instances observed in this clinic sarcoid cases with negative tuberculins have recovered completely and the tuberculins have remained negative.

DISCUSSION

The results in this small series of cases suggest that patients with positive tuberculin tests give false positive tests with lepromin. This problem has been studied in great detail by Fernandez in Argentina (3, 4, 5). Fernandez (4) found that the tuberculin test and the Mitsuda reaction (lepromin?) agreed in ninety-seven per cent of the tests done on individuals apparently free of leprosy and coming from countries where there is no leprosy. In another study one hundred and twenty-three children with negative Mantoux and Mitsuda reactions were inoculated with B. C. G. antituberculin vaccine. One month later the tuberculin reaction was positive in 99.18 per cent and the Mitsuda in 91.86 per cent.

The most striking clinical differences between the positive tuberculin and the positive lepromin test are the delayed onset, slow development and slow decline of the lepromin test. In the tuberculin the antigen is in solution while the antigen or antigens in the lepromin material are still bound in the bodies of the bacilli, and the delay in the appearance of the test could be explained as the time required for the tissue fluids to dissolve the particulate antigens.

SUMMARY AND CONCLUSIONS

Ten patients with Boeck's sarcoid were tested with lepromin. Negative tests were obtained in seven and positive ones in three. Six of the seven negative tests were in patients known to have negative tuberculin tests, and two of the three positive were in patients with positive tuberculins. Two patients with active tuberculosis gave positive lepromin tests. Apparently patients with positive tuberculin tests give false positive tests with lepromin. No evidence was obtained to indicate that Boeck's sarcoid is an attenuated or modified form of leprosy.

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THE REACTION TO LEPROMIN OF PATIENTS WITH SARCOID OR TUBERCULOSIS COMPARED WITH THAT OF PATIENTS IN GENERAL HOSPITALS WITH A DISCUSSION OF THE MECHANISM OF THE REACTION[1]

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The possibility that generalized sarcoidosis of Boeck may be caused in some instances by Mycobacterium leprae has been suggested by the clinical and pathologic resemblance of certain cases of sarcoid to tuberculoid leprosy. Other observers have commented on the difficulty of making a differential diagnosis between these two conditions, particularly in cases where the skin is extensively involved (1). Some investigators still question whether Mycobacterium leprae has been recovered by culture or animal inoculation (2). The failure to recover constantly from patients with sarcoid any etiologic agent by culture on a wide variety of media or by inoculation of many species of animals would therefore be consistent with a relationship to tuberculoid leprosy (3). Acid-fast stains of scrapings from the nasal mucosa or from sarcoid nodules and of slides prepared from excised tissue have been negative, but organisms cannot always be demonstrated by this technique even in tuberculoid leprosy. The present study was undertaken to determine by means of an immunologic or biologic reaction whether sarcoid is actually related to leprosy, or whether lepromin can be used for differentiation.

The lepromin test consists in the intradermal injection of an antigen prepared from a boiled, ground saline suspension of excised lepromatous tissue rich in Mycobacterium leprae (4). It has been variously called the lepromin, the leprolin, and the Mitsuda test. The classical reaction is delayed in its appearance and is read at 21 days. The cause of the variations in the reaction occurring in known cases of human leprosy needs further interpretation. Because of these variations, the lepromin reaction is considered of no value in diagnosis but is useful in determining the prognosis and in classifying cases of leprosy.

Since almost all individuals in certain areas of the tropics where leprosy is endemic give a positive reaction to lepromin, it was necessary to establish a baseline for this study by testing normal individuals in a non-endemic area of this country (5). Lepromin has not been widely used in the United States; no data are available on the incidence of reactions in non-endemic areas, and the data accumulated on the incidence of reactions in endemic areas are not yet available.

Some investigators believe that sarcoid is anergic tuberculosis; therefore, patients with tuberculosis were also tested with lepromin.

MATERIAL

The lepromin was obtained from the United States Public Health Service, through Dr. G. H. Faget, Medical Officer in Charge, U. S. Marine Hospital, Carville, Louisiana (Leprosarium). Acid-fast bacilli were demonstrated in the antigen by staining a drop of the material with carbolfuchsin by the Ziehl-Neelsen technique (fig. 1). Normal saline was used as a control.

A small amount of antigen prepared by Dr. V. Pardo-Castello of Havana, Cuba, was furnished us by Dr. David T. Smith of Duke University, Durham, N. C., for comparison with the Carville antigen. This material on stain also showed acid-fast organisms.

Tuberculin was obtained from the Forsyth County Tuberculosis Sanatorium and from the Saranac Laboratory, Saranac, New York. The tuberculin control solutions furnished by each were used.

Seventy patients were tested with lepromin. These included 5 patients with sarcoid seen in their homes; 6 patients with active tuberculosis who were confined in the Guilford County Tuberculosis Sanatorium, Jamestown, N. C.; patients with healed or inactive (apparently cured) tuberculosis and individuals suffering from a variety of

[1] Read at the 40th annual meeting of the American Society of Tropical Medicine at St. Louis, Missouri, November 16, 1944.

The cost of reproducing the illustrations and tabular material was generously borne by The American Foundation for Tropical Medicine, Inc.

chronic diseases—41 patients in all—at the Forsyth County Hospital, Winston-Salem, N. C.; and 18 patients with a variety of acute and chronic diseases, including 1 with active tuberculosis on the wards of the North Carolina Baptist Hospital, Winston-Salem, N. C. The diagnosis of tuberculosis or sarcoid as made by the sanatoria[2] was accepted without question.

FIG. 1. Photomicrograph of lepromin antigen stained by the Ziehl-Neelsen technique, showing clumps of beaded, acid-fast bacilli. An oil immersion lens was used.

(We are indebted to Dr. Wiley D. Forbus and Mr. Carl Bishop, Department of Pathology, Duke University School of Medicine, for the photomicrographs.)

METHOD

All injections were made in the skin of the flexor surface of the forearm with tuberculin syringes. New syringes and needles were obtained for the skin tests and were not used for any other purpose.

[2] We are indebted to Dr. Paul Yoder for furnishing us a list of patients with sarcoid diagnosed at the Forsyth County Sanatorium, and to Dr. M. D. Bonner for permitting us to give the test to patients with tuberculosis at the Guilford County Sanatorium.

One syringe and needle was always used for the same test solution, and the syringes and needles were never interchanged. After each use they were washed with distilled water only, placed in marked glass tubes, and sterilized in an autoclave.

Because the findings in the tuberculous group suggested a cross reaction with lepromin, tuberculin tests were done simultaneously with the lepromin tests on all patients in the latter part of the experiment.

Lepromin was injected in a dose of 0.1 cc. Tuberculin and control solutions were freshly made up with normal saline to a dilution of 1:1,000, and 0.1 cc. (0.1 mg.) was injected. In some instances 0.1 cc. of a 1:100 dilution (1.0 mg.) was used.

In the tuberculous and control groups the lepromin reaction was observed at 20 minutes, and at

1, 3, 7, 14, 21, 40, and 56 days following the injection. The readings on patients with sarcoid were done at 20 minutes and 1, 3, 10, 17, 25, 31 and 40 days. The reaction was recorded in the manner suggested by Faget, following Hayashi (4). Infiltrated areas 0.3 to 0.5 cm. in diameter were classed as 1 plus; those 0.5 to 1.0 cm., as 2 plus; those 1.0 cm. and over, and those areas showing necrosis or pus, as 3 plus. Erythema without induration occurring at 24–48 hours was noted as suggested by Fernandez and recorded by the same scale as the tuberculin reaction (6). The tuberculin reactions were read at 24 and 48 hours and recorded by the usual scale, ranging from 1 to 4 plus.

Biopsy specimens were taken from 4 normal individuals—1 on the fifty-second day and 3 on the fifty-ninth day. Biopsies were done on tissue taken from 1 patient with inactive tuberculosis on the fifty-second day, and from 2 patients with active pulmonary tuberculosis on the eighth day. The biopsy sections were fixed in 10 per cent formalin or in Bouin's solution and were stained with hematoxylin and eosin. Acid-fast stains were done on some sections.

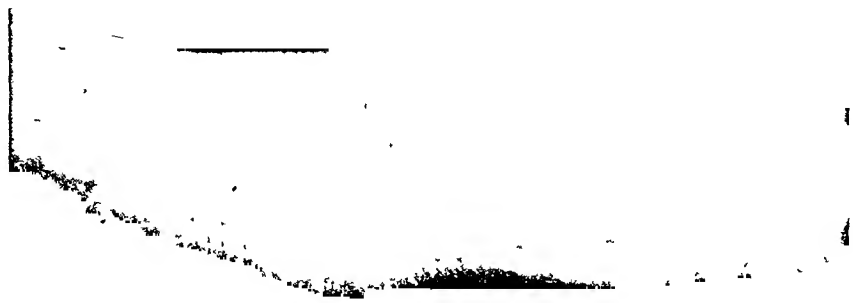

FIG. 2. Photograph of slight (1 plus) lepromin reactions at 24 days in a patient with a negative tuberculin reaction. The upper reaction is to American antigen and the lower to Cuban antigen.

RESULTS

General

Sixty-three out of 70 patients (90%) gave at least a 1 plus reaction at some time (fig. 2); 41 patients (56%) gave 2 or 3 plus reactions at some time. One plus reactions were frequently noted, occurring in 29 instances early and in 15 late. Because of the small size of such reactions and their failure to fit any definite pattern, they were considered of doubtful significance. In the composite analysis 1 plus lepromin reactions are grouped with the negative reactions in one set of data; in the other, 1 plus reactions are grouped as positive with 2 and 3 plus reactions (table 1).

Age, sex, and color did not appear to affect the results; hence the detailed data on these matters are not included in the charts.

Time

In order to simplify the composite analysis, reactions occurring at 1 to 3 days were grouped as early, those at 3 to 20 days as medium, and those after 20 days as late (table 1). Only 3 instances of immediate reactions at 20 minutes with wheal and erythema were encountered, all in the control group. Only 8 patients who developed early reactions (fig. 3) failed to develop late classical reactions (fig. 4). If 1 plus reactions are included, 53 patients were positive early and 52 were positive late. Of those with 2 and 3 plus reactions, 24 were positive early and 37 late.

Disease

Sarcoid: Of the 5 patients with sarcoid, 3 gave 1 plus reactions at some time. None gave 2 or 3 plus reactions (table 2).

Tuberculosis: Six of the 7 patients confined to sanatoria with active or recently arrested tuberculosis showed 2 or 3 plus reactions to lepromin at some time; the remaining patient was in the terminal stage of far advanced active tuberculosis (table 3A). The reaction increased in intensity with increasing time. Five of the 9 patients who showed 3 plus reactions were in the tuberculous group; this fact is especially striking because of the small size of the group.

Part B in table 3 shows the results of lepromin tests in patients who were apparently cured of tuberculosis and who had remained well for intervals of two years or longer. The diagnosis was made on the basis of a recent x-ray examination or of a known history of tuberculosis with definite diagnosis and confinement in a sanatorium. The reactions to lepromin in this group tended to be less marked than in the active or recently arrested group.

Miscellaneous diseases (control group): In the control group of 58 patients with various diseases 23 gave early reactions of 2 to 3 plus, 6 tests were positive at the medium time, and 31 gave positive late reactions (tables 4 and 5).

TABLE 1

*Composite results of Lepromin tests**

CASES	DISEASE	LEPROMIN + - +++			LEPROMIN ++ - +++		
		Early (precoz)	Medium	Late (classical)	Early (precoz)	Medium	Late (classical)
		Days 1-3	4-20	21-56	1-3	4-20	21-56
5	Sarcoid	2	2	2	0	0	0
7	Tuberculosis, active	1	6	7	1	2	6
58	Control, total	50	32	43	23	6	31
24	Control, Tbcln. unknown	21	9	15	12	2	12
34	Control, Tbcln. known	29	23	28	11	4	19
16	Known Tbcln. 0 - +	13	6	10	5	0	6
18	Known Tbcln. ++ - ++++	16	17	18	6	4	13
26	Known Tbcln. 0 - ++	22	15	20	9	3	13
8	Known Tbcln. +++ - ++++	7	8	8	2	1	6

By the calculation of standard errors it can be shown that the total control group is homogeneous whether the reaction to tuberculin is known or unknown. There is no significant difference in the frequency of positive lepromin reactions between the group in which the reaction to tuberculin is known and that in which it is unknown.

By the calculation of standard errors the comparison of the tuberculous group with either control group is significant with regard to 2 and 3 plus lepromin reactions at the late time (Diff./S.E. = 2 2). Because of the small number of cases involved the tuberculous group cannot be compared with the sarcoid group. The results in the sarcoid group as compared with the control group are probably significant, though the zero in the late group of 2 and 3 plus reactions leads to a statistical fallacy.

* In order that the results of the lepromin tests in various disease processes may be compared, the sarcoid group from table 2 is not included in the control group with known tuberculin reactions.

FIG. 3. Photograph of early or precoz moderately intense (2 plus) lepromin reaction at 24 hours in a patient with a 4 plus tuberculin reaction. The tuberculin reaction is on the left, the lepromin reaction on the right.

Comparison with tuberculin

Because our results suggested that tuberculosis might increase the lepromin reaction, 34 patients with various chronic diseases in the control group and 5 patients with sarcoid were tested simul-

taneously with tuberculin and lepromin. For this statistical analysis, all patients on whom tuberculin tests were done were included (table 5). Twenty-five of the patients were tested with 1:1,000 tuberculin (table 5, part A) and 14 with 1:100 dilution of tuberculin (table 5, part B). In the summary (table 5), so that various degrees of reaction to tuberculin can be compared with various degrees of reaction to lepromin, 2 plus tuberculin reactions are grouped first with 3 and 4 plus tuberculin reactions and then with the negative and 1 plus reactions. One plus reactions to lepromin are considered positive in the first set of data, and are omitted in the second. Statistical analysis by the 2 by 2 table and chi square methods strongly suggests a correlation of 2, 3 and 4 plus tuberculin reactions with 2 and 3 plus lepromin reactions at 21 days. The trend is statistically significant when 1 plus lepromin reactions are included as positive.[3]

Comparison of American and Cuban antigens

Since a similar study was independently and simultaneously being conducted by Dr. Kenneth D. Weeks and Dr. David T. Smith, using lepromin antigen obtained from Cuba, a small amount of this material was tested in 13 patients (7). No essential difference in activity between the American and Cuban antigens was noted when they were simultaneously injected into the same patients.

Biopsies

Biopsy specimens taken from the lepromin reaction in patients with active tuberculosis showed a response which was qualitatively similar to that in normal individuals but quantitatively more pronounced. The cells were chiefly large mononuclears, epithelioid cells, fibroblasts and many lymphocytes. Neutrophils and eosinophils were few. Giant cells of the Langhans type were present in tuberculous patients. No tubercles or lesions readily recognizable as leprosy were seen (figs. 5, 6 and 7). No acid-fast material was found after appropriate staining, but serial sections were not done. The histopathology of the early lepromin reaction is well described by Fernandez (6)

FIG. 4. Photograph of a strongly positive (3 plus) late classical lepromin reaction at 24 days in the same patient shown in figure 3. The tuberculin reaction on the left shows scaling and a small crust (4 plus); the lepromin reaction on the right above (to American antigen) shows a small crust (3 plus); in the reaction on the right below (to Cuban antigen) necrosis was minimal (3 plus).

TABLE 2

Lepromin reaction in sarcoid

CASE	TBNCL.	DAYS						
		1	3	10	17	25	31	40
1	0	+	0	0	0	+	+	
2	++++	0	0	+		+	+	
3	++	+	+	+	+	0	0	
4	+++	0	0	0	0	0		
5	0	0		0	0	0		0

DISCUSSION

Incidence

It is apparent from this study that unequivocal lepromin reactions may be less frequent in a nonendemic area of the United States than in endemic areas in the tropics. Among 70 patients with a variety of acute and chronic diseases, 41 (56%) showed unequivocal (2 or 3 plus) reactions at some time. A comparison of these results with those obtained in areas in the United States where leprosy is endemic, such as Texas, Louisiana

[3] We are indebted to Dr. Nash Herndon for statistical criticism of our results.

TABLE 3

Lepromin reaction in tuberculosis

CASE	ACTIVITY	CLASSIFICATION	TBCLN.	DAYS 1	3	7	14	21	30	40	56
A											
1	Active	Far advanced		0	0	0	+	++	+++	+++	
2	Active	Far advanced		0	0	0	+	+	+	+	
3	Active	Far advanced	*	0	0	++	+	+	+++	+++	
4	Active	Moderately advanced‡		0	0	+	+	++	+++	+++	
5	Active	Minimal		++	+	0	0	+		++	
6	Arrested	Far advanced		0	0	+		+++	+++		
7	Arrested	Far advanced		0	0	++	++	+++	+++	+++	
B†											
8	Apparently cured	Lymphoid	++++	+++	0	+	+			++	++
9	Apparently cured	Minimal	+++	+		+				++	+
10	Apparently cured	‡		++	++	++	++			++	++
11	Apparently cured		++				+			++	++
12	Apparently cured		+	+	0	+	0			0	+

* Sputum positive at time of test.

† Group B is taken from cases in tables 4 and 5.

‡ Fibroid type.

TABLE 4

Lepromin reaction in various diseases, tuberculin unknown

CASE	DAYS 1	3	7	14	21	40	56
1	0	0	0	0	0	0	
2	0	0	0	0	0	0	
3	0		0	0		0	0
4	+	0	0	0	0	0	
5	+	0	0	0	0	0	
6	+	0	0	0		0	
7	+	0	0	0	0	0	
8	+	+	0	0	0	0	
9	+	+	0	0		0	0
10	++	+	0	0	+	++	
11	+	+	+	+	+	+	
12	+	+	+	+	+	+	
13	+	+	+	+		++	+
14	++	++	+	+	+	+	
15	++	+	0	0	+	++	
16	++	+	0	+	++		
17	++	+	0	0	+	++	
18	++	+	0	0	+	++	
19	++	+	0	0		++	
20	++		+	+		+++	+
21	++	++	++	++	++		
22	++	++	++	++		++	++
23	+	++	+	+		+++	++
24	+++	0	0	0		+++	+++

Summary

LEPROMIN	DAYS 1–3	DAYS 4–20	DAYS 21–56
+ - +++	21	9	15
++ - +++	12	2	12

and Florida, would be extremely helpful in interpretation. No data in these areas are available (8).

Significance of slight reactions

It is difficult to compare the incidence of 1 plus reactions in this series with results in the tropics. Little attempt has been made in reports from other areas to differentiate between slight, moderate and intense (1, 2, and 3 plus) reactions. Because of the small size of the reaction and the absence of signs of inflammation, we believe that the slight (1 plus) reactions are of little significance and should be regarded as non-specific foreign body reactions. This point will be discussed more fully under the possible mechanism of the reaction. We believe that only 2 or 3 plus reactions should be considered significant. This view is in agreement with that of Eccles, who has worked with a similar antigen in an endemic area of the United States (9).

The relation of sarcoid to leprosy

Since none of the small group of patients with sarcoid gave more than slight (0 to 1 plus) reactions, no etiologic relationship between Mycobacterium leprae and sarcoid has been demonstrated. These results are in complete agreement with those of Weeks and Smith, who simultaneously conducted a similar study in 10 patients with sarcoid; negative reactions to lepromin antigen were obtained in 7 instances and positive reactions in 3 (7). Neither of these studies produced

TABLE 5

Lepromin compared with tuberculin A—1:1,000; B—1:100

CASE	TBCLN.	DAYS 1	3	7	14	21	30	40	56
A 1	0	0	0	0	0			0	0
2	0	+	0	0	+			+	+
3	0	+		0	0			++	+
4	0	++		+				0	0
5	0	++	0	0	0			++	+
6	0	++	++	0	0			+	+
7	0	++	+	+	0			++	0
8	0	++	0	0	0			++	+
9	+	+	0	+	0			0	0
10	+	+	0	+	0			0	+
11	+	+	+	0	0			++	+
12	+	+	0	0	+			++	++
13	++	+	0	0	0			+	0
14	++	+	+	+	+			+	+
15	++	+		+	+			++	+
16	++	+		++	+			+	+
17	++	+		+	+			++	+
18	++	++		+	+			++	++
19	++	++	+	++				++	+
20	++				+			++	++
21	++	++	++	++	++			++	++
22	+++	+	0	+				++	+
23	+++	+		+				++	+
24	+++	+++	+++	+++	+++			++	++
25	++++	+++	0	+	+			++	++
B 26	0	0	0	0	0	0	0		
27	0	0	0	0		0		0	
28	0	+	0	0		0	0		
29	0	0	0	0		+	+		
30	0	+	0	0	0	+	+		
31	+	0	+	0	0	0	0		
32	++	+	+	+	+	0	0		
33	++	+	++	0	0	+	++		
34	+++	0	0	0	0	0			
35	+++	+	0	+	+	++	++		
36	+++	+	0	0	+	0	+		
37	++++	0	0	+		+	+		
38	++++	0	0	0	0	0	+		
39	++++	+	+	+	+	+++	+		

Summary*

TUBERCULIN	CASES	DAYS 1–3 Lepromin + - +++	X^2	DAYS 1–3 ++ - +++	X^2	DAYS 4–20 + - +++	DAYS 4–20 ++ - +++	DAYS 21–56 + +++	X^2	DAYS 21–56 ++ - +++	X^2
0 - +	18	14	.06	5	.003	6	0	11	4.77	6	3.17
++ - ++++	21	17		6		17	4	19		13	
0 - ++	29	24	.7	9	.45	15	3	21	1.3	13	.69
+++ - ++++	10	7		2		8	1	9		6	
Total	39	31		11		23	4	30		19	

Statistical analysis by the X^2 method indicates significant correlation of tuberculin and late lepromin reactions when 1 plus tuberculin reactions are considered negative, and 1 plus lepromin reactions are considered positive. The same trend is clearly indicated when 1 plus late lepromin reactions are grouped with negative reactions.

* The 5 patients with sarcoid in table 2 are included here because tuberculin tests were done on them. The results are recorded in the column which most closely corresponds to the day noted in table 2.

evidence that sarcoid is attenuated or modified tuberculoid leprosy. Fernandez has found early and late positive lepromin reactions in 89 per cent of 71 patients with tuberculoid leprosy (6). The lepromin reaction would therefore seem to be useful in the differential diagnosis of sarcoid.

possible that the body becomes sensitized to some gradually liberated fraction and then reacts as additional organisms are destroyed in the body and more sensitizing antigen is released?

The observations that lepromin reactions were more intense in patients with active or recently

FIG. 5. Photomicrograph of a biopsy specimen taken seven days after injection from a 2 plus lepromin reaction in the skin of a colored male with active pulmonary tuberculosis. ×107.

Mechanism of the reaction in relation to tuberculosis

Little is known of the mechanism of the lepromin reaction, but the presence of acid-fast organisms in the antigen is known to be essential. The increase in the number of positive reactions after the second week would seem to indicate that the mechanism of the classical late lepromin reaction is not comparable to that in the commonly used skin tests (such as the tuberculin, Shick, Frei), where the reactions occur in 5 days or less. The delay of days or weeks suggests that the lepra bacillus must be broken down by the body before the active chemical fractions are liberated. Is it

arrested tuberculosis and that in these patients they increased in intensity with increasing time suggest that a cross reaction between the two infecting organisms exists. Mycobacterium leprae and Mycobacterium tuberculosis have common staining qualities and hence probably some similar chemical characteristics. The apparent correlation between positive classical lepromin reactions and positive tuberculin reactions also suggests the possibility of a biologic cross reaction in the host between some similar antigens in these organisms.

The early or precoz reaction described by Fernandez, occurring at 24 to 48 hours, was observed

(6). In general, the early reaction paralleled the late classical (21 day) lepromin reaction in its relation to the tuberculin test, though the similarity was much closer if 1 plus reactions are included. The early reaction was infrequently observed in patients with active pulmonary tuberculosis in our series, but this group was small. In studying the early reactions, Fernandez used unfiltered lepromin antigen as well as the soluble substances remaining after the removal of the bacterial bodies by filtration. No attempt was made in our study to separate soluble antigens or haptens by filtration. It is known that Mycobacterium tuberculosis contains several soluble antigens, including polysaccharides (carbohydrate) and proteins. Mycobacterium leprae probably contains similar antigens, which would go into solution if the intact organisms were autolyzed or mechanically broken up as by grinding. McKinley has discussed in some detail possible cross reactions occurring in lepers between the soluble antigens—chiefly proteins without polysaccharides—of Mycobacterium tuberculosis and other acid-fast organisms. Some of the antigens employed in his study were obtained from cultures of "leprosy bacilli," which may or may not have been the true Mycobacterium leprae (10).

Hayashi has reported that 67 per cent of 826 lepers, regardless of the classification of the leprosy, gave positive tuberculin reactions (4). Fernandez has reported that in lepers the lepromin and tuberculin tests frequently give divergent results (12).

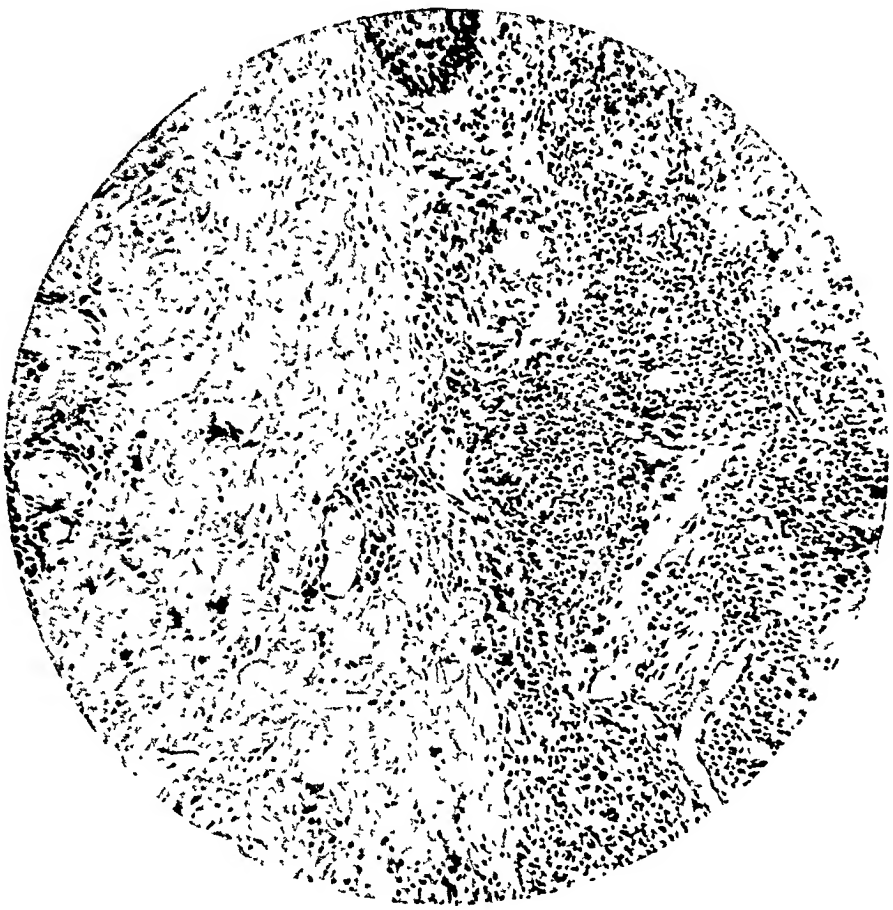

FIG. 6. Photomicrograph of a biopsy specimen taken 62 days after injection from a 1 plus lepromin reaction in the skin of a white female who had a 2 plus tuberculin reaction. ×107.

Fernandez has further reported that of 26 patients who were free of leprosy but who had skin tuberculosis or who had been treated intensively with B. C. G. (avirulent tubercle bacillus) vaccine, 20 had 2 or 3 plus late classical lepromin reactions and 3 had 1 plus late reactions (11). In this group, 7 patients had 1 plus tuberculin reactions (1:1,000) and 10 had 2 plus reactions,

but only 5 had 3 plus reactions. In persons coming from non-endemic countries and supposedly free of leprosy, the lepromin and tuberculin tests gave the same reactions in 97 per cent of 42 cases; 91 per cent of the patients had positive reactions to tuberculin (12). Fernandez also studied 193 healthy children in an orphanage. Of 54 with positive tuberculin tests, 45 reacted positively to lepromin; of 139 who had no reaction to 0.1 mg. of tuberculin, only 5 reacted to lepromin (12) These facts would indicate that the antigen common to the two organisms is not a protein similar to that found in tuberculin.

Fernandez has inoculated with B. C. G. vaccine a group of 123 children who had negative tuberculin and lepromin reactions. One month later the tuberculin reaction was positive in 99 per cent and the lepromin in 92 per cent (12). This experiment confirms the suspicion that the common antigen is not a protein; apparently it is present in the whole bacillus and can sensitize the patient.

The early reaction of tuberculous patients to the filtrate of lepromin observed by Fernandez suggests an allergic response to a soluble antigen. A similar but slightly more prolonged reaction has been observed in tuberculous patients by one of us (Harrell), using a purified lipid fraction of Mycobacterium tuberculosis, a phosphatide which contains carbohydrate in its molecule; we believe that the carbohydrate is liberated in the body as the phosphatide is destroyed (13).

The early reaction to water soluble fractions of lepromin, presumably containing easily liberated carbohydrate or protein, will not explain the delayed classical lepromin reaction. The prolonged reaction with delayed necrosis which results from the injection of a second purified lipid fraction of

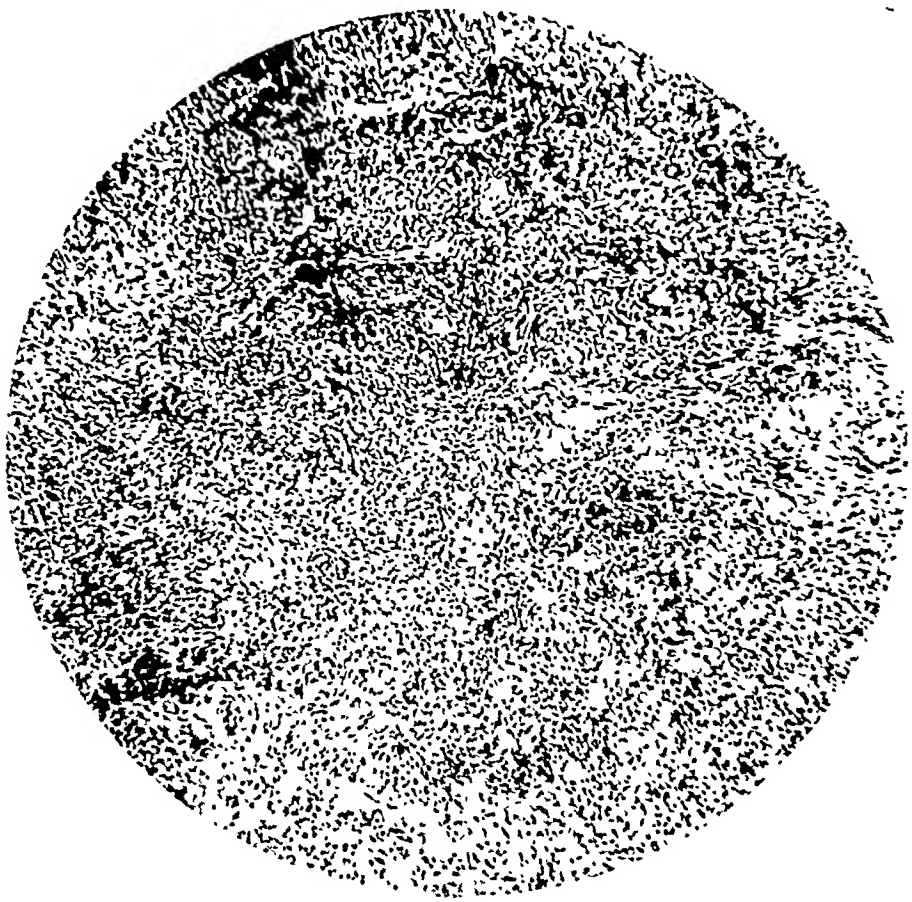

FIG. 7. Photomicrograph of a biopsy specimen taken at 62 days after injection from a 2 plus lepromin reaction in the skin of a white female with apparently cured calcified glandular tuberculosis who had a 4 plus tuberculin reaction. ×107.

the tubercle bacillus, a wax, suggests that one slowly acting fraction of lepromin is a similar complex lipid (13). The reaction to the tubercle bacillus wax was thought to be non-specific, since it occurred also in patients with diseases other than tuberculosis. The increased intensity and frequency of late reactions to lepromin in patients with tuberculosis suggest that a lipid or the breakdown product of a lipid may be common to both Mycobacterium leprae and Mycobacterium tuberculosis.

The mononuclear reaction in tissues is common to leprosy, sarcoid and tuberculosis. The response to soluble polysaccharide fractions of acid-fast organisms is usually polymorphonuclear in type. The response to protein, particularly in the presence of allergy, may be eosinophilic or polymorphonuclear or it may be chiefly in the form of plasma cells (14). The response to lipids is usually mononuclear, though we have observed eosinophilic reactions in some instances. A comparison of the cellular response to lipids with that observed in the biopsies of the lepromin reaction (figs. 5, 6 and 7) again suggests that a lipid is the common factor in the monuclear response observed in leprosy, sarcoid and tuberculosis. The quantitative alteration in the response of tuberculous patients suggests an altered reactivity on the part of the patients to some fraction of the organism, probably a break-down product. The number of cases studied is too small for any definite conclusions to be drawn, but the absence of inflammatory or allergic response after 48 hours and the presence of a type of large mononuclear cell suggest that the lipid is a complex one of the nature of an acetone-soluble fat or wax.

Diagnostic value

Why are reactions to lepromin so frequent in endemic areas? Some may be due to cross reactions with tuberculosis, as has been discussed already; further study may show that lepromin could be used for the diagnosis of certain forms of leprosy in the tropics in patients who have no demonstrable tuberculosis. It seems unlikely, however, that tuberculosis is sufficiently prevalent in the tropics to account for the very high incidence of positive lepromin reactions.

It is usually said that leprosy is a disease of low contagion, requiring long exposure, but that, when once contracted, it is of relatively high virulence—low curability. This theory will not explain why children of lepers who are not separated from their parents soon after birth develop within one year positive lepromin reactions, even in the absence of clinical or laboratory evidence of infection (15). The presence of a positive lepromin test in these children in the absence of demonstrable infection indicates that the disease is one of high contagion but relatively low virulence—high curability. Regular exposures to small numbers of Mycobacterium leprae for a short time may be more important in the pathogenesis of the disease than haphazard exposures over a considerable length of time. These facts are true of tuberculosis and may be equally true of leprosy.

If the mechanism of the lepromin reaction suggested above is correct, it is probable that neither the protein nor the carbohydrate fraction of the tubercle bacillus could alone be used to rule out tuberculosis so that the classical lepromin reaction could be used for the diagnosis of leprosy. Additional studies of early reactions should be made and the carbohydrate and protein fractions of mycobacterium tuberculosis should be compared with filtrates of lepromin presumably containing similar antigens. For clarification of the mechanism of the late classical reaction additional chemical study of the purified lipids of Mycobacterium tuberculosis in relation to purified lipids of Mycobacterium leprae should be made, if the latter organism can be recovered in sufficient quantities to be subjected to chemical analysis, such as that described by Anderson (16). McKinley has tested the phosphatide, leprosin, and leprosinic acid fractions of a supposed strain of Mycobacterium leprae (10).

Lack of an adequate control solution

At the present time the control solution used in the performance of the lepromin test is not adequate. Saline may detect a highly irritable skin which would give false positive early reactions, but this solution is useless as a control for the late classical reaction. A control for the delayed response should be composed of some material which must be broken down slowly over days or weeks. Suspensions of tuberculous tissue derived from guinea pigs have been used, but these introduce possible error through reactivity to foreign animal protein. Suspensions of tubercle bacilli have been used by Cummins in studying the mechanism of the lepromin reaction, but the possibility of a lipid fraction common to the two

organisms makes such a suspension unsuitable as a control (17). Perhaps a suspension of timothy bacillus, a non-pathogenic, acid-fast organism, might be satisfactory. The chemical analysis of Mycobacterium leprae should indicate compounds which are known to be non-reactive, and are sufficiently similar to fractions of the bacillus to be used as controls.

Suggested methods for the recovery of Mycobacterium leprae

The mononuclear reaction in tissues is common to leprosy, sarcoid and tuberculosis. In lepromatous leprosy the organisms are found in mononuclear cells, and in large numbers. Does the living monocyte furnish a substance essential for the growth of Mycobacterium leprae? It is known that viruses and rickettsias grow only in living tissue cells and cannot be recovered on artificial media. Therefore, attempts should be made to recover Mycobacterium leprae from lesions by inoculation of infected material into tissue cultures of monocytes, or by injection into chick eggs, probably the chorio-allantoic membrane.

Why have animal inoculations failed to produce progressive disease? Must repeated injection of organisms be given in order to mobilize sufficient monocytes to insure a fertile soil? From the accepted facts concerning the epidemiology of leprosy it is apparent that repeated exposures over an appreciable period of time are necessary to produce the disease. Experiments with animals have consisted chiefly in single inoculations of infected material. It may be necessary to give daily inoculations over a period of months in order to build up an infecting dose of organisms. Such methods for recovery of the organisms will more closely approximate the conditions under which the infection is acquired naturally.

SUMMARY

1. In patients with sarcoid, reactions to lepromin were infrequent and slight; no indication was found that sarcoid is atypical tuberculoid leprosy.

2. Slight (1 plus) lepromin reactions are of doubtful significance.

3. The incidence of intense or moderately intense lepromin reactions in a control group of persons in a non-endemic area of the United States is less than that in endemic areas of the tropics.

4. Strongly positive late lepromin reactions occurred in patients with active pulmonary tuberculosis.

5. The high incidence of intense or moderately intense lepromin reactions in persons with strongly positive tuberculin tests suggests the presence of common sensitizing antigens.

6. A possible mechanism based on soluble protein or polysaccharide antigens is suggested to explain the early lepromin reactions observed in persons with positive tuberculin reactions.

7. The possibility is suggested that the mechanism of the late lepromin reactions in persons with positive tuberculin tests, or with active pulmonary tuberculosis, may be related to insoluble, firmly bound lipid fractions which would be slowly liberated by destruction of Mycobacterium leprae in tissues.

8. By comparison with the cellular response to purified lipid fractions of Mycobacterium tuberculosis, the mononuclear response to lepromin observed at biopsy further suggests a lipid as the active chemical fraction.

9. If tuberculosis is ruled out by a suitable control substance, lepromin may prove useful in the diagnosis as well as the prognosis of leprosy.

10. No adequate control substance for interpretation of the late lepromin reaction has yet been introduced.

11. Leprosy may be, like tuberculosis, a disease of high contagion, slow progression, and high curability, requiring repeated regular exposures to the infecting organisms.

12. Attempts should be made to recover Mycobacterium leprae by inoculation of tissue cultures of monocytes or of chick egg chorio-allantoic membrane with infected material, since pathologic evidence suggests that the living monocyte furnishes a substance essential for growth of the organism. Attempts to reproduce the disease should also be made by frequent inoculations of living organisms into animals over a long period of time.

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